

nature

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Are summit agreements of much use?

WHATEVER the outcome of top-level meetings that heads of state seem to delight in, the agreements on scientific, technical and medical cooperation can always be relied on as a stand-by. If the discussions were cordial, wide ranging and in an atmosphere of mutual understanding then there were probably plenty of good things to emerge: grain deals, trade credits, non-aggression treaties and the like are bound to eclipse anything on the scientific side. But if the discussions were merely correct, a helpful exchange of views and in an atmosphere of mutual respect, then scientific agreement is wheeled out as a major symbol of that mutual respect, and some hare-brained joint projects are launched. Part of the deal often consists of trips for the presidents of the respective academies around endless laboratories of their counterpart's country. Few scientists have not had at some time or other to conduct an immensely bored (with great justification) scientific luminary on a fifteen minute tour as the result of some cosmetic deal concluded ages before by heads of state.

Mercifully, Mr Wilson returned from Moscow last week with a bagful of deals to present to the public, so we were spared Anglo-Soviet cancer projects, joint expeditions to the South Pole and so on. And yet although not eye catching, there are agreements of interest to the scientist, although you'll have a job finding out much about them, as not many people in Whitehall seem to know very much about what went on.

Perhaps the most bizarre idea to emerge was of a round-table conference of academics. Apparently someone (Harold Wilson, one suspects) thought it a good idea to put together a diversity of 'distinguished representatives of public life, science, culture, commerce, the press and other fields' from the two countries. When in Britain they will meet under the auspices of the Royal Institute for International Affairs, which thought it a bit premature to say anything other than that the concept has not progressed very far yet. How this strange group will be 'selected', what it will talk about and what good will be done by it has yet to be spelt out. We shall follow its career with the mildest of curiosity.

An almost equally outrageous idea that found its way into the communique was that the Soviet Union and Great Britain would collaborate on attempts to keep to a minimum the number of underground nuclear tests; this is no doubt meant to be seen as a positive gesture by two of the three guarantors of the Non-Proliferation Treaty.

Since the British government has got out of the habit of acknowledging to the public its nuclear activities and since the Soviet government never got into the habit in the first place, they have both, in a sense, already kept things down to a minimum. Unless these two arch rivals start sharing their top-secret data, it is hard to see how the commitment is other than pure humbug.

On scientific and technological cooperation *per se*, the communique does little beyond pat on the back two inter-governmental commissions the existence of which we had all but forgotten. The commission on applied science, technology, trade and economic relations comes up with encouragement for joint efforts in (among other things) high temperature plasma physics, astronomy and applied microbiology, although it is difficult to see much rhyme or reason in the list.

What do scientists really want from meetings of heads of state? Nobody seems to ask the community before visits, and certainly it is hardly the easiest thing to find out to whom one should talk if one feels strongly. It is perhaps foolish to look for anything from bilateral talks; science, as opposed to big technological projects, is nominally an international affair. Nonetheless, there are some matters of principle that could have been raised. If science is indeed international and one works in a spirit of trust that transcends frontiers, accessibility is the key to scientific development. Scientists tend to rely fairly heavily, in developing their own ideas, on the work of those other scientists whom they either know and have visited or at least whom have heard favourably about from a colleague.

On the whole it is possible to form these contacts almost without limit in many countries, and the better the telephones, airlines and travel budgets the better the opportunities for doing science. The links are palpably inadequate in the Anglo-Soviet situation; exchanges of a few scientists a year do little more than scratch the surface, and the ultimate aim must clearly be to make an Anglo-Soviet agreement as redundant as an Anglo-American one would be. Neither the recent agreement, nor any envisaged in the near future, goes very far in this direction.

If you have any ideas and you don't think you will be invited to the round-table, it is a bit of a problem knowing whom you should contact. Try the International Technological Collaboration Unit at the Department of Trade.





Counting the Common Seal

How many Common Seals are there in the Wash? Is the population in danger of extinction? Conflicting estimates of the size of this stock have attracted considerable public attention since the Home Secretary refused to grant hunting licences in 1974 in spite of the fact that 400 could legally be culled under the Conservation of Seals Act 1970. Dr C. F. Summers of the Seals Research Division (SRD) of the Institute for Marine Environmental Research and Dr M. D. Mountford of the Institute of Terrestrial Ecology, both of the Natural Environment Research Council, explain the basis on which population estimates are made.

THE seal biologist does not have access to samples representative of whole populations in the same way that a fish biologist might, because commercial sealing in Britain concentrates almost exclusively on seal pups which provide high quality skins for the fur trade. It is, therefore, difficult to estimate the size of seal stocks directly.

This problem is usually surmounted by measuring some index of the population, such as the number of pups born annually. This is useful for identifying trends in stock size and, if adequate life-table data for the species are available from another source, it is possible to convert pup production to an all-age estimate (assuming that the life table parameters have similar values in both stocks).

If we consider the problem of obtaining an estimate of annual pup production, the Common Seal, *Phoca vitulina*

vitulina, presents peculiar difficulties. Unlike the Grey Seal, *Halichoerus grypus*, it does not congregate into recognisable breeding assemblies, nor do the pups remain on land for more than a few hours after birth. Direct counting of pups does not, therefore, yield realistic estimates of production. Nevertheless, this is all that can be attempted in some localities and the best information obtainable is a minimum estimate of productivity.

In the Wash, however, where pups are relatively easy to catch for tagging and where the commercial hunters have cooperated by returning the tags, it has been possible to obtain a more realistic estimate of pup production than elsewhere. Before describing this it is interesting to consider earlier attempts to measure the size of the Common Seal stock in the Wash.

Counts of Common Seals hauled out

on sandbanks throughout the Wash have been recorded since the 1920s. Before 1965, however, the counts did not cover the entire area. They were made by individual observers from boats and, even with a very fast boat, it is not possible to visit all the sandbanks in the Wash during the period of a single low tide.

Since 1965 aerial photographic surveys have been made by the SRD at all states of tide and throughout the year, visiting all the sandbanks in a short period of time. Fluctuations in the counts made from these photographs suggest that the isolated counts of a few sandbanks made from a boat cannot be used to estimate the size of the whole population. Moreover, since it is not known what proportion of the population is hauled out at any one time the published counts from the aerial photographs do not give a correct estimate of the number of seals in the Wash. It was suggested in the 1972-73 report of the Universities Federation of Animal Welfare that only a small proportion of seals remain in the water at low tide and that as the maximum number of seals reported hauled out at any one time was 1,722 (July 17, 1969) the population size of around 6,000 reported by the SRD must be a gross overestimate. This argument is invalidated by the very large variation in the aerial counts. For example, in February 1968, 472 seals were counted as compared with 1,534 in March 1968. Between these two observations the population had either trebled through immigration, or a very large proportion of the seals which hauled out in March did not do so in February.

Common Seals shot in the Wash between 1911 and 1953 attracted a bounty paid by the Ministry of Agriculture, Fisheries and Food and the Eastern Sea Fisheries Committee. The animals were killed to protect local fisheries and consisted of juveniles and adults. Commercial seal hunting for the fur trade started in the Wash in the early 1960s and large numbers of pups were taken before the passage of the Conservation of Seals Act 1970. (The Act provides protection for both Grey and Common Seals during their respective pupping seasons but also allows seals to be killed for several different purposes. Seals are killed under licence in the Wash in compliance with that section of the Act which permits "the use of a population surplus of seals as a resource.")

Between 1968 and 1973, the SRD used a capture-recapture technique to estimate Common Seal pup production in the Wash. Pups were tagged in June and early July and an estimate of pup numbers was made from the proportion of tags 'recaptured' in the hunters'

catch, usually in the second and third weeks of July. To give a correct estimate the capture-recapture method requires certain conditions to be met. (1) The tagging and the hunting effort should be random. Older pups are more difficult to tag and shoot, but the chance of being tagged and the chance of being shot, if related at all, must be positively correlated. This has the effect of underestimating the population size.

(2) In the interval between tagging and hunting there must be no gains or losses through immigration, emigration or mortality. Immigration and emigration are not important, since pups move only short distances during the first few weeks of life. If, however, this condition is not met with regard to mortality, the capture-recapture method overestimates the population size by a factor of $1/p$, where p is the probability that a pup, alive at the time of tagging, is still alive and present in the population at the time of hunting. No precise figure of the mortality during the sampling period is available but a 21% post-natal mortality for the first year of life has been reported for *P. vitulina richardi*. Therefore, the upper limit of the degree of overestimation that could be produced by the capture-recapture method is given by assuming that the whole of this first-year mortality occurs in the interval between tagging and hunting. If this happened the method would overestimate the population here by about 27%.

(3) There must be no recruitment of pups during the 'recapture' sampling. Although this is not the case, while the method overestimates the population at the time of tagging, it gives an unbiased estimate at the time of hunting.

(4) There should be no difference in mortality between tagged and untagged pups. Since it was found that tagging did not affect the growth rate it is unlikely to have increased pup mortality.

Table 1 shows the pup production estimates calculated for the years 1968-73. There is no value for 1969 because tags were not properly returned, or for 1974 because there was no hunting. When the five years are combined, an estimate and approximate 95% confidence interval of $1,450 \pm 239$ pups is obtained. The data suggest neither an upward nor a downward trend in productivity.

Using a life-table for *P. vitulina richardi* in British Columbia compiled by M. A. Biggs (*Bull. Fish. Res. Bd Can.*, No. 172, 1969) it is evident that the number of pups born represents about 1:4.5 of the total stock. The estimate obtained from this table for the all age population, that is pups,



Cow with pup

non-breeding and breeding adults, is $6,525 \pm 1,076$.

We have referred to a possible overestimation by the capture-recapture technique but, considering other available evidence, it is fairly certain that during the mid-1960s the Wash population was at least 4,000. In 1966, 850 pups were shot by commercial hunters. As it is unlikely that they ever shot an entire year class, the all-age population must have been in excess of about 3,825 (850×4.5) in 1966. This level of hunting would take four or five years, after recruitment of survivors to the breeding stock, to influence pup production. But whatever its effect the population size in 1970 could not have been less than about 3,915 ($4.5 \times$ number of pups shot, see Table 1) and, adding the same proviso about the hunters taking an entire year class, was probably much higher.

Our population estimate is considerably larger than the maximum number of seals counted hauled out together (1,722 on July 17, 1969). Juvenile Common Seals from the Wash have been reported from the Forth estuary to the French coast in tag recoveries. If they occur on any appreciable scale, these movements from the area, perhaps to feed, would mean that a proportion of the total stock was missing from the

Wash haul-outs at any particular time. Local population 'explosions' at places such as nearby Donna Nook, Blakeney and Scroby Sands cause alarm among drift-net fishermen but last for a few days only and suggest that such feeding migration often occur.

The Common Seal in the Wash is clearly not on the verge of extinction as some reports have claimed. In the early 1970s there was a conviction among biologists and seal hunters alike that the heavy exploitation of the Common Seal in Shetland, where around 90% of the annual pup production was taken, would irreversibly reduce the stock if allowed to continue. Consequently a complete ban on Common Seal hunting in Shetland was implemented in 1973. The level of exploitation in the Wash has never reached this high figure. Nevertheless, the provisions of the 1970 Act allowed the introduction of a more restricted hunting regime. The quota was set at 400 pups in 1971, compared with an average of over 700 a year in the six-year period immediately preceding the passage of the Act. The current legislation provides a flexible means of applying controls as they are needed and as long as there is a programme of continuous monitoring, the stock in the Wash should not be at risk. □

Table 1 Capture-recapture data and estimates of pup production

Year	No. of tags applied	No. of tags in catch	Size of catch	Estimate of pup production
1968	37	12	459	1,309
1970	39	22	870	1,419
1971	174	27	303	1,900
1972	111	35	380	1,175
1973	193	36	380	1,987

international news

Less cash for British science

by Eleanor Lawrence

THE Science Budget recently announced for 1975-76 by the Chairman of the Advisory Board for the Research Councils (ABRC), Sir Frederick Stewart, holds little joy for the research councils. There is still a fall in total expenditure of 1% on the figure for 1974-75 and all the research councils except the Social Science and Natural Environment Research Councils will have less money in real terms compared with this year. Even more telling is the observation that the level of spending for 1975-76 represents 6% less than expectations of a few years ago.

The total of £171 million, approved by the Secretary of State for Education and Science but still to be passed by Parliament, is split up into £13.1 million for the Agricultural Research Council (ARC), £28.9 million for the Medical Research Council (MRC), £19.2 million for the Natural Environment Research Council (NERC) and £96.4 million for the Science Research Council (SRC). The Social Science Research Council receives £8.7 million and the remainder goes in grants to the Royal Society and the Natural History Museum.

The ARC, the MRC and the NERC also receive substantial commissions from the appropriate government departments, under the terms of the 1972 White Paper.

But, after the present period of contraction, the ABRC is proposing a period of growth up to 1980, albeit at the modest rate of 3.5% a year for the MRC, the ARC, the NERC and the SSRC, and only 0.7% for the SRC. This reflects the feeling that the time has come to look more closely at the proportion of Science Budget taken up by 'big science' projects (in astronomy and high energy physics for example) which need costly equipment and installations



SMALL MEANS MORE

that will be used by only relatively few scientists. The Mark VA radiotelescope has already been vetoed and the SRC will now have to decide whether it can afford two other projects, the Northern Hemisphere Observatory (estimated to cost £12 million) and the major accelerator project (EPIC) estimated to cost around £25 million.

The ABRC's view is that, in these uncertain times, overall scientific capability should be maintained even at the expense of some of the SRC's prestigious and intellectually exciting

big science. Also, with the decrease in university funding the research councils may find themselves having to pick up bigger bills for the multitude of small projects in universities financed by research council grants but with facilities provided by the university.

Even the cautiously optimistic forward look of the ABRC could fall foul of present and forecast economic crises. The call of certain politicians for decreased public spending generally could erode even the proposed modest rate of growth. □

Annual percentage growth of the Science Budget and research councils' allocations

	1966-67	1967-68	1968-69	1969-70	1970-71	1971-72	1972-73	1973-74	1974-75	1975-76	1976-77-1979-80
ARC	7.3	12.2	7.3	3.2	7.0	3.1	3.7	4.0	-3.7	-0.6	3.5
MRC	11.6	14.9	5.1	6.9	8.1	3.6	3.6	2.7	-1.7	-1.0	3.5
NERC	17.8	20.6	13.5	20.4	10.4	5.3	4.9	4.4	-3.0	+0.2	3.5
SRC	13.2	6.9	5.4	2.9	3.8	4.8	4.1	3.2	-2.2	-2.2	0.7
SSRC	—	47.3	47.9	25.9	30.3	16.0	10.0	8.6	5.7	+6.8	3.5
Total	13.3	11.2	7.4	6.1	7.1	4.7	4.2	3.9	-2.1	-1.3	2.2

Transfers to departments under the terms of the 1972 White Paper have been included in figures for after that date.

THE full effect of the cuts that President Ford wants to make in federal support for biomedical research has now been calculated by budget officials at the National Institute of Health (NIH). When stated in terms of the number of projects that would go unfunded if Congress allows Ford to have his way, the NIH figures make the situation look horrendous.

The background is that at the end of January President Ford proposed to slice \$351 million from the budget that Congress has already approved for the NIH for the 1975 fiscal year (which is now two-thirds over). Then he followed that suggestion with a budget proposal for the NIH next year which would reinstate only \$72 million of the cut. Congress has to approve both those measures before they can be put into effect, however, and it is unlikely to do so.

One reason is that at the funding level that Congress has already approved for the NIH this year, only about 49% of the projects submitted for NIH support and approved as scientifically worthwhile by peer-review groups would actually be funded. Even that would represent a decline of 4% from last year, but if the proposed cuts are made, only 29% of the approved grants would be funded. And, at the budgetary level proposed for next year, the proportion would sink to a miserable 18%.

That situation arises because a good deal of the NIH's budget is already earmarked to support projects begun in previous years but which still have some time to run. In other words, the axe would fall heaviest on those projects which are now coming up for support, which means that few new ideas would get funded, and biomedical research would be put into something of a holding pattern.

Moreover, if the proposed cuts are made, even continuation grants would be reduced by 5%—a figure which the Administration insists can be absorbed without having to terminate any project, but which will amount to about 13% or 14% when inflation is taken into account.

Stated in term of absolute numbers, the situation is no less depressing. According to NIH figures, at the budgetary level approved by Congress, the NIH could fund about 4,360 competing projects this year (that is, new projects and those coming up for grant renewal) but at the level proposed by President Ford, only 2,320 competing projects would get the green light. For the National Cancer Institute alone, the difference between the Congressionally approved budget and President Ford's proposal amounts to about 620 projects—about 1,200 competing grants would be funded at the Congressionally approved level whereas only 580 would

get the green light under Ford's proposed budget.

With those figures to cogitate upon, Congress, which has a history of being more generous than the Administration toward the NIH, is very unlikely to allow the cuts to be made.

● Meanwhile, in spite of huge uncertainties in the funding of biomedical research, the number of students enrolling in graduate biology departments continues to increase dramatically. According to the latest compilation of figures from the National Science Foundation (NSF), graduate enrolment in the life sciences shot up 17.2% last autumn, and thanks to that surge in numbers, total enrolment in science and

Washington seen

by Colin Norman

engineering courses at graduate schools throughout the United States is higher now than at any time since 1969. Demand for most non-biological courses remained static, however, the only other popular field being the social sciences where enrolment rose by about 4.4%.

The sharp upturn in the number of students taking graduate courses in the biological sciences follows a period of rapid growth in undergraduate biology departments in the past few years. When asked by the NSF if they could explain this fascination with biology, deans and chairmen of biology departments suggested that part of the reason is that many students who fail to get into medical school because of fierce competition are taking up related fields of study instead. Another oft-cited reason is that biology offers a way to "make a contribution to society", the NSF study notes.

Whatever the reason, the upturn in numbers of graduate students in science and engineering departments is welcome news for the universities, which have recently been in dire financial straits. And it is also worth noting that this year's huge increase in enrolments in graduate biology departments has come at a time when many biomedical scientists have been warning that cut-backs in federal support for the biological sciences would dissuade bright young scientists from taking up a career in biological research.

Furthermore, the NSF figures suggest that the number of students taking up engineering courses may have decreased slightly this year, a fact which does not augur too well for the future supply of engineers to carry out the promised expansion of energy research and development in the United States.

● Nearly seven months after atmos-

pheric scientists began to warn that the propellants used in aerosol spray cans could deplete the ozone layer, the federal government has announced that it has established a high level task force to look into the problem. Although slow off the mark, the government is now hoping to move quickly—the task force has been given only four months in which to produce a report.

That will, however, be no easy matter, for the task force has been given a very broad remit. It is expected to "summarise atmospheric, medical and ecological information on the subject, evaluate possible economic impacts and alternatives available to the industry, define potentially applicable authorities under which federal actions can be taken, and outline the proposed federal program to resolve the issue". The task force will, at least, have a wealth of data available from the recently published report on the effects of supersonic aircraft flying in the stratosphere.

At the same time, the National Academy of Sciences has a study in progress on the effect of aerosol spray propellants—fluorochlorohydrocarbons, which are usually referred to by the trade name of Freons—on the ozone layer. The chairman of that study group is Dr Donald M. Hunten, of Kitt Peak Observatory, who has already said at a press conference that in his opinion, the injection of Freons into the atmosphere should be halted as rapidly as possible and the moratorium on their use should last at least until the dangers have been fully evaluated.

Concern over the atmospheric effect of Freons is focused on a theory, published in *Nature* last year by Drs M. J. Molina and F. S. Rowland, of the University of California at Irvine, that the propellants will break down in the upper atmosphere to release chlorine, which will then attack the ozone layer. Because ozone filters out much of the harsh ultraviolet radiation from the Sun, it provides an important protective shield around the Earth; threats to the layer's composition should therefore not be taken lightly.

The chief problem with assessing the effect of Freons on the ozone layer is that because they are chemically very inert, they will drift upwards very slowly, remaining unchanged until they reach the upper atmosphere. Because it may take years for Freons to reach the ozone layer, even if their use is halted as soon as any effect is noticed, depletion would continue for several years after a ban is imposed.

That is one reason for the short time that the task force has been given. Another is that the Consumer Product Safety Commission is being sued by a public interest law firm to ban aerosol spray cans. □

Materials shortage shocks ahead?

by Colin Norman, Washington

The National Academy of Sciences committee report forecasting a bleak future for fossil fuels in the US (February 20) was not limited exclusively to the supply and demand for oil and gas. "Man faces the prospect of a series of shocks of varying severity as shortages occur in one material after another", it suggests, and adds that "the first real shortages (are) perhaps only a few years away."

The overriding conclusion that runs through the 348-page report (prepared by COMRATE—the academy's Committee on Minerals Resources and the Environment) is that more effort is needed to conserve materials and to improve the efficiency with which they are used. "Above all", it states, "we should adopt a conservation ethic that has at its heart avoidance of waste and more efficient use of materials."

● **Copper.** Although deposits of copper within the United States are, in theory, sufficient to meet demand at least until the end of the century, the report notes that its availability will be dictated by success in finding new deposits and that there is also likely to be a shortage of smelter capacity. COMRATE therefore concludes that "we do not foresee continued United States self-sufficiency in production of primary copper and therefore recommend a strong policy of conservation". The only large, unexploited deposits of copper so far identified are manganese nodules on the deep seabed, and the report recommends that "developing the recovery of copper and associated metals from these nodules be encouraged with due regard to the potential impact of undersea mining on the environment". Surprisingly, however, the report has virtually nothing to say on the touchy political question of how exploitation of manganese resources should be regulated—an issue which sharply divided the Law of the Sea Conference last year, and according to most American mining companies, until the political questions are settled at an international level the prospects for deepsea mining are cloudy, at best.

● **Substitutes** need to be found for three materials which have unique uses, and whose supply is threatened, COMRATE suggests. They are helium, which is likely to be in high demand for use as a coolant for superconductors, particularly for transmission lines; mercury, which has no substitute for its use in mercury-wetted switches, and asbestos.

As far as helium is concerned, the



THE 1975 campaign of the Health Education Council to warn pregnant mothers of the dangers of smoking has just begun. Mr Alastair Mackie, the council's Director-General, claims "we do not want to increase the usual anxiety felt during pregnancy . . . [The campaign] exploits the success

Is it fair to force your baby to smoke cigarettes?



This is what happens if you smoke when you're pregnant.

Every time you inhale you fill your lungs with nicotine and carbon monoxide.

Your blood carries these impurities through the umbilical cord into your baby's bloodstream.

Smoking can restrict your baby's normal growth inside the womb. It can make him underdeveloped and underweight at birth. Which, in turn, can make him vulnerable to illness in the first delicate weeks of his life.

It can even kill him. Last year, in Britain alone, over 1,500 babies might not have died if their mothers had given up smoking when they were pregnant.

If you give up smoking when you're pregnant your baby will be as healthy as if you'd never smoked.

achieved last year when research showed that behavioural change followed our advertising. We learned useful lessons, and these will be put into effect in the new project". Above (left): new project; right: the old. Below: new copy. No prizes for spotting a 'useful lesson' learned.

When a pregnant woman smokes she puts her unborn baby's life at risk. Every time she inhales, she poisons her baby's bloodstream with nicotine and carbon monoxide. Smoking can restrict your baby's growth inside the womb. It can make him underdeveloped and underweight at birth. It can even kill him. In just one year, in Britain alone, over 1,500 babies might not have died if their mothers had given up smoking when they were pregnant. If you give up smoking when you're pregnant your baby will be as healthy as if you'd never smoked.

The Health Education Council

report recommends that conservation should be encouraged and that alternatives be found for such applications as arc welding and dispersive uses such as purging rockets and spaceships.

● **COMRATE** further suggests that alternatives be found for chromium, gold, platinum and palladium since those metals are largely concentrated in a few countries, thereby opening up the possibility that the United States could become vulnerable to embargoes or international cartels. It singles out chromium in particular, since virtually all the world's resources are concentrated in Rhodesia and South Africa. In addition, the report suggests that substitutes be found for the use of tin in view of potential world-wide shortages of the metal. The report emphasises, however, that "the discovery and development of new and improved materials as possible substitutes for existing ones takes time and . . . the process is generally driven by clearly perceived functional objectives rather than by ill-placed optimism that 'something will turn up when the crunch comes'".

● **Coal.** Although the report notes that there should be no shortage of coal supplies in the United States for more than a century, it has a few complaints

to make about the adequacy of research and regulations designed to ensure that coal is mined and used more carefully.

As far as mining is concerned, the report suggests that there is an "urgent need" to overhaul methods for detecting, diagnosing and compensating for pneumoconiosis, the so-called Black Lung Disease which claims the lives of thousands of miners, and which in terms of compensation alone may cost up to \$8,000 million a year by 1980.

The report also urges that efforts to mitigate the health hazards associated with the use of coal be stepped up. In particular, it is recommended that more research be conducted on the health effects of small particles, and on the growing problems of acid rain caused by the oxidation of sulphur dioxide to sulphuric acid. As a matter of priority, the report also urges that equipment for removing oxides of sulphur—so called stack gas scrubbers—should be installed in plants where high sulphur fuels are burnt. Several electric utilities, most notably the American Electric Power company, have been fiercely resisting such requirements and have been conducting advertising campaigns running into millions of dollars to try to win public sympathy for their case. □

AFTER a year notable for set-backs, the Soviet space programme has made a successful start to 1975 with the Soyuz-17/Salyut-4 mission. As well as testing out some modifications to the hardware, notably the new autonomic navigation system, the two-man team carried out a wide range of biological and physical experiments.

Although some of these experiments are now of a fairly routine nature—medical tests on the cosmonauts and monitoring the ultraviolet radiation of the Sun, for example—some of them have a more adventurous quality. Among these must be classed the 'Filin' experiment, designed by a team from the Shternberg Astronomical Institute of Moscow State University. This comprises a group of spectrometers for monitoring X-radiation of stellar origin in the 0.2–10 keV range, together with telescopes with a field of view of approximately 1°, intended to correlate the spectrometer readings with visual observations. It is hoped that the results of this project may provide information on white dwarfs and neutron stars. Special attention was given to Rigel and to the remains of the Vela supernova. A somewhat speculative *Pravda* article (January 24) suggests that this experiment may even throw some light on the vexed subject of black holes. During one orbit simultaneous infrared and X-ray observations were made of the characteristics of the background radiation along the Galactic meridian.

Nearer home, the 'Spektr' experiment studied the ambient conditions of the spacecraft, and in particular, the effect of its constant bombardment by charged particles. A special apparatus, produced by one of the Instrumentation Institutes of the Space Research Institute of the Soviet Academy of Sciences, consisted of two subsystems—one to analyse the incident flow of particles and the other to analyse those emitted after impact. These measurements, which were recorded on tape so as not to overload the communications channels to ground control, are intended, not only for use in plotting a quantitative model of the upper ionosphere, but also for the design of future spaceships and orbital craft.

Solar observations, now a routine part of any orbital mission, included a series of spectrography and diffraction spectrometry experiments, which automatically recorded the ultraviolet radiation from the entire solar disk, while at the same time flight engineer Grechko photographed sunspots and prominences.

Later investigations with the solar telescope included an experiment in which protective coatings were

deposited on the surface of the mirrors, in order to assess the possibilities of creating and restoring good optical properties to mirrors subjected to bombardment erosion.

Another routine experiment—the recording of infrared radiation from the Earth and Moon—on this occasion included a special "deep-cooling"

Soviet spacemen's programme

from Vera Rich



Grechko and Gubarev board Soyuz-17.

system "sublimation cold accumulator", to maintain the sensitive element of the infrared telescope-spectrometer at the required temperature. These infrared observations of Earth always form an important part of Soviet orbital missions, since they can be justified in terms of the national economy as a means of surveying resources. In the Salyut-4 programme, however, infrared observations were also used to investigate cosmic phenomena. Salyut 4 carried a special infrared device, the ITS-K, which, in the words of Dr M. N. Markov of the Lebedev Physics Institute of the Academy of Sciences,

can "at the same time seek the 'finger-prints' of the molecular components of matter on the planets and can also look under definite layers of the atmosphere surrounding the planets of the Solar system" (*Pravda*, January 30, 1975). The ITS-K experiment, in fact, is a continuation of a programme begun by the Lebedev Physics Institute, back in 1968, using high altitude (500 km) rocket observations.

Visual photography (both black-and-white and colour) is also traditionally presented by the planners as a valuable contribution to "the study of the environment and the solution of individual problems in the interests of the national economy". On this mission, special emphasis has been placed on such photography, since, in general, the study of such photographs taken in winter has been somewhat ignored. It is hoped that the snow-cover may throw into strong relief ravines and gullies and other features of geological interest. Special attention has been given to the photography of Soviet Central Asia and Kazakhstan, and the south-western European region of the USSR including the Caucasus. Unfortunately for the conservationists, weather conditions prevented photography of the Baikal area.

The on-board experiments (code-named 'Oasis') included a number of genetic, embryological, physiological and biotechnical projects using insects, microorganisms, tissue cultures and higher plants. Special mention is made of *Drosophila* (once legislated out of Soviet science under Lysenkoism) and *Chlorella*. Biological experiments on the cosmonauts themselves included a series of tests monitoring the respiratory and cardiovascular systems using graded loads, a "special vacuum suit" and a rotating chair. On the nineteenth day of the flight, an ultrasonic device was used to determine changes in the density of bone tissues, and blood samples were taken for post-flight analysis.

Also at the biological level, considerable importance was laid on the recycling of water (from atmospheric moisture), to be used by the cosmonauts for drinking and food preparation.

At the beginning of this mission, it was stressed by Major-General Georgii Beregovoi, Head of the Cosmonaut Training Programme, that it should not be considered simply as a preparation for the forthcoming Soyuz-Apollo project. Nevertheless, when the Soviet team for the project flew to Houston on February 8, for the next round of preparatory talks, there can be little doubt that both sides saw the success of the Salyut-4 mission as a promising augury for the joint venture.

Will the US make its own laws of the sea?

by Colin Norman, Washington

Two bills which are now being pushed independently in the United States Congress are likely to have a significant impact on the second session of the Law of the Sea Conference, which opens in Geneva next month. One measure is designed to protect the financial interests of American companies involved in mining operations on the deep sea bed, and the other would extend the jurisdiction of the United States over fishing rights from 12 to 200 miles. Both those issues were at the centre of intense debate during the first session of the Law of the Sea Conference held in Caracas last year, and the prospect of unilateral American action on either of them is certain to colour debate in Geneva.

As for the issue of mining on the sea bed, there was virtual deadlock in Caracas between developing nations, which generally supported the idea of establishing an international authority to regulate commercial operations and research on the sea bed, and industrialised nations, which were reluctant to give an international body so much authority over commercial enterprises. The importance of the bill which was introduced into Congress last week by Senator Lee Metcalf, a Democrat from Montana, is that it signifies that if the conference deadlocks again, the United States may well go ahead with regulation of deep sea mining on a unilateral basis.

Although Metcalf's bill was introduced last year and Congress failed to pass it, it should be regarded more seriously this time because Metcalf has made a deal with some powerful committee chairmen which will ease the bill's progress through the Congressional mill. The clear inference being given by Metcalf's staff is that if the Law of the Sea Conference deadlocks, the bill will be out of committee and on the floor of the Senate very quickly.

The impact of the bill on the Geneva conference should also be seen in the light of the recent claim on an area of the sea bed filed by Deepsea Ventures Inc., one of the leading companies involved in exploratory work on the mining of manganese nodules. Although the claim, which was filed in the United States in November last year, has not been recognised by the State Department, it signifies that Deepsea Ventures is prepared to press ahead with its mining operations no matter what happens at the Law of the Sea Conference.

Metcalf's bill, in short, would estab-

lish a licensing scheme, operated by the Department of Interior, which would issue exclusive licenses to American companies for prospecting rights on large blocks of the sea bed. Later, the companies would be able to apply for licences to begin commercial exploitation of minerals on the sea bed. Although the bill states that the licensing operation would be replaced by any mechanism that may be established by international treaty, the Administration has consistently opposed the measure on the grounds that it would pre-empt discussions at the Law of the Sea Conference.

Last year, the bill was passed by the Senate Interior Committee soon after the Law of the Sea Conference ended in Caracas, but the Senate Foreign Relations Committee claimed jurisdiction over the measure and kept it bottled up until Congress adjourned. This year, however, Metcalf has concluded a deal with the chairmen of both the Armed Services and the Foreign Relations committees which would give them only 30 days to consider the bill once it is passed by the Interior committee. At the end of that period, the bill would automatically go on the Senate calendar and be brought to a vote.

One other factor could influence the situation later this year. The Interior Department, which has consistently opposed Metcalf's bill, is now developing legislation of its own which will be introduced as an alternative measure if the Law of the Sea Conference is inconclusive. The bill, which was first brought to light by *Ocean Science News*, a newsletter well versed in the affairs of the marine science industry, would provide for prospecting licences to be issued to American corporations but would impose a moratorium on commercial exploitation.

As for the fisheries bill, a measure supported chiefly by Senator Warren Magnuson, a powerful Democrat from Washington State, was passed by the Senate late last year, but it was not considered by the House. Magnuson intends to re-introduce the measure this week and the House Committee on Merchant Marine and Fisheries will be holding public hearings during the second week of March on a similar measure.

The opposition of the Department of Defence was considered an important factor in preventing the bill from being passed by the full Congress last year, but there have recently been rumours—which the Department of Defense will neither confirm or deny—that the department no longer opposes the measure. If that is the case, Congress could well pass a fisheries protection bill while the Law of the Sea Conference is still in session. □

Fighting disease on the Volta River

from Peter Collins

THE development of new techniques for applying pesticides is being intensively studied by the Centre for Overseas Pest Research (COPR) as part of Britain's contribution to the vast onchocerciasis ('river blindness') control project now getting under way in the Volta River Basin. This project, sponsored by the World Bank, the UN Development Programme, and the World Health Organisation (WHO), is expected to cost some \$200 million over the next 20 years, with financial and technical cooperation from Canada, France, West Germany, the Netherlands and the USA, in addition to about £500,000 from this country as an initial contribution.

The opening phase of the project, managed by the WHO, aims to eliminate the black fly, *Simulium damnosum*, which is the vector of the parasite directly responsible for the disease, and which can only be effectively attacked during its larval stages. These are aquatic; the project thus involves a massive use of pesticides in the Volta rivers and their many tributaries in which the flies breed. It is here that the WHO has sought the help of the COPR, whose experience of tropical pest control was built up during the many years of its predecessor, the Anti-Locust Research Centre. In the present project, the problem is one of applying larvicides to many hundreds of miles of rivers running largely through dense forest, and of ensuring that they reach the larvae, which live in the fast-running stretches attached to sub-aqueous vegetation or to the river bottom, where they feed by filtering material brought down by the current.

Early trials, carried out by the COPR under contract to the WHO and in cooperation with a French onchocerciasis team, showed that the normal system of spraying from fixed-wing aircraft or helicopters was unsuitable or ineffective, partly because a large proportion of the pesticide is inevitably wasted on riverside vegetation. To avoid this, Cliff Lee, of the COPR, devised a method for dropping quantities of pesticides *en bloc* at a suitable distance upstream of the fast-flowing stretches favoured by the *Simulium* larvae. The prototype 'rapid release' device now being used in field trials is designed to apply accurately measured quantities of from 0 to 50 litres in a single drop, and has so far given extremely good results. The formulation devised for Abate (the pesticide chosen by the WHO as least likely to damage other aquatic species) is such

LAST month Madame Simone Weil, minister of health, declared that "the Pasteur Institute is no longer able to survive with only the help of its own resources," and deplored the fact that the prestigious institution "had been run by scientists who did not always possess the qualities of administrators."

Now the financial situation of the Institute is being examined by the *Cour des Comptes*, the government's accounting agency. When this report is completed, Madame Weil is to meet the institute's administrators and representatives of the scientific personnel in an attempt to find a solution for the Pasteur Institute "to continue its work in favourable conditions, either through increases in government subsidies, or by the State taking over some of its expenses."

The objective, says the dynamic and popular minister of health, "is to come to a point where the Pasteur does not have to keep asking for an amount of money it needs. A coherent and continuous system must be set up to allow the Institute to find its equilibrium." The Ministry of Industrial and Scientific Development, and that of Education, are also involved in the discussions.

This latest *crise* is one of many that have beset the Pasteur Institute since 1967, when a severe financial crisis triggered the so-called "Pasteur Institute revolution," which was followed by the replacement of a number of ageing administrators by scientists, and the drafting of new statutes providing for the separation of research and production activities.

As the new president of the institute's board of administrators, Jacques Monod attempted to maintain the Pasteur's status as a private institution (although one playing a vital national role) and to balance its books. He introduced into the hallowed walls of the building where Pasteur had carried out his experiments, a down-to-earth businessman, Jean Hardy, who came from the food industry.

Arrangements had to be made for some of the institute's products to be marketed by the pharmaceutical industry, and this gave rise to awkward situations. For instance, the Laboratoires Roger Bellon held a license to sell some of the Pasteur Institute's vaccines and biological products. Roger

Bellon was then purchased by Rhône-Poulenc, which also happens to have a controlling interest in the Institut Mérieux, which was founded by a disciple of Pasteur and is the Pasteur Institute's most successful commercial competitor.

Pasteur's progress

from Alexander Dorozynski, Paris



Monod: devoting himself to research

Two years ago, Institut Pasteur Productions, S.A. officially came into being, and took over commercial activities under the financial direction of Jean Hardy. Professor Monod agreed to head IPP temporarily as well as the Institute before returning entirely to scientific activities. It turned its profits over to the Pasteur Institute, but in spite of an annual production of some 17 million doses of serum and vaccines as well as of assorted biological testing products, and of substantial income from abroad from the licensing of the "prospective influenza vaccine" developed at Pasteur two years ago, ends wouldn't meet. The dream (which was defined by Professor Elie Wollman, associate director of the Institute, as that of a "socialist society of scientists, supported by a capitalist structure") just did not work out. The Pasteur got deeper into the red, and started eating up some of its capital, most of which came from the investment of donations of 2.5 million francs made at the time of the institute's founding in 1888 by contributors such as the Tsar of Russia, the Sultan of Turkey, and the Emperor of Brazil.

As a non-profit research organisation attempting to finance itself through a

commercial subsidiary, it remained in a difficult position. There is little doubt that if IPP had decided to go into outright competition with the profitable French pharmaceutical industry, chances of success should have been good. But marketing "Pasteur Institute Aspirin" or other current drugs under the Pasteur label was not in the tradition. "We are not a drug company," said Prof. Wollman. "We don't sell drugs and I don't expect we shall."

Towards the end of last year, Professor Monod proposed his radical "renovation plan": to move away from the original location of the institute in the city (abandoning the laboratories, the research hospital, the historical library, as well as the grave of Pasteur), sell the 50,000 square metres of valuable real estate for some 250 million francs, and move to Garches, a few miles north of Paris, where the institute has the use of a large estate and has some production facilities.

The plan was opposed by a vast majority of the research staff, led by François Jacob. Last month Professor Monod resigned his post as president of the directors "to devote himself fully to stimulating scientific research at the institute". He was replaced by "businessman" Jean Hardy, who remains at the same time director of Pasteur Productions.

It has been pointed out that Dr Monod's return to scientific activity has been planned long ago, but perhaps it was triggered by a feeling (shared by some members of the Ministry of Health) that an outstanding scientist is not necessarily a good administrator.

At the same time a department of corporate development was created, now headed by Joël de Rosnay, a scientist with several years' experience in industry.

The Pasteur's problem is not so much to survive—that it undoubtedly will—but to survive while keeping its independence from state control and *functionarization*. This independence has not paid off in cash terms, but it seems to have paid off in terms of research. If the awarding of Nobel prizes is a criterion of value, the argument is a strong one; out of nine Nobel prizes awarded to Frenchmen in the fields of medicine and biology, eight have been given to *pastoriens*. □

that it spreads rapidly on the surface of the stream, forming what has been described as "an almost monomolecular film". At the same time, it is found effective as far downstream as 50 kilometres from the point of release, which is some 200 metres above the first site to be dealt with in any one drop. This is in strong contrast to the range of the same material when

applied by orthodox spraying techniques some 15–80 kilometres downstream.

The prototype equipment, designed by the COPR and made by CIBA-Pilatus for the WHO, was intended for use with fixed-wing aircraft and the first trials were carried out with a normal light aircraft. It has now been adapted for use with the helicopters to which the WHO is at present com-

mitted on this project, partly because many of the rivers wind for miles through narrow walls of dense forest, where fixed-wing aircraft cannot safely be used. But on the larger and more open stretches, especially where sites to be treated are far apart, light aircraft may well prove more economical because of the much greater distances that they can cover. □

correspondence

Soviet dissenters in need of help

SIR,—The case of Vladimir Bukovsky (February 6) highlights a disturbing aspect involving my own profession. Bukovsky was released from a labour camp in 1970 after several spells of 'treatment' for his dissenting views. He spent the next 14 months of liberty collecting evidence of psychiatric abuses for the suppression of political dissent in the Soviet Union at great risk to himself. His only hope of protection lay in the forthcoming Congress of the World Psychiatric Association (WPA) in Mexico City in 1971. It was to psychiatrists in the West, and to the psychiatrists attending this congress in particular, that Bukovsky sent his covering letter in the form of an appeal when he sent the documents out to the West. Their authenticity has never been questioned. The Executive Committee of the World Psychiatric Association declined, however, to allow discussion of this problem despite the efforts of some psychiatrists. This shameful act of betrayal produced a savage reprisal in the form of a 12-year prison sentence and the terrible conditions under which Bukovsky has to exist. It is not a story that a psychiatrist can tell with pride.

We now have information that the Soviet authorities were extremely apprehensive about the Mexican Congress. At the time Mr Victor Fainberg, a dissenter, was in a Leningrad psychiatric prison hospital, from where he has now succeeded in emigrating to Israel. He tells me that he and his friend Borisov made certain demands for improving the conditions of the inmates of the hospital and for a re-examination of their own cases by the Court. Prior to the Mexico Congress conditions improved and the Soviet authorities agreed to have the cases re-examined. As soon as the decision of the Executive Committee of the World Psychiatric Association became known, conditions became worse than they had ever been and their request for re-examination by the Courts was dismissed with a contemptuous laugh. It can be assumed that in other special hospitals the same thing occurred. The failure of the WPA to rally to the defence of Bukovsky and the other 'lunatic' dissenters was an act of opportunism and devoid of all compassion.

I am writing to urge psychiatrists to do all they can to right a terrible wrong



Bukovsky: appealed to psychiatrists

that has been committed and to put all possible pressure, both individually and through their associations, on their Soviet colleagues and Soviet authorities to secure the release of a brave and generous man.

To move to the case of another dissident whose case has been mentioned in *Nature* recently, I can report that I have been asked by Mrs Plyushch to act as psychiatrist in an investigation into her husband's psychiatric state. This is in connection with legal action which she intends to bring against the Dnepropetrovsk special hospital for unlawful detention and criminal negligence in his treatment at that hospital.

Leonid Plyushch was sentenced to detention in a special hospital after examination by three psychiatric commissions, one of which pronounced him sane. The final one, presided over by Professor Snezhnevsky considered him as suffering from schizophrenia and stated that he had 'delusions of inventions', a reference presumably to Plyushch's interest in games theories, which he shares with his wife.

The case was heard *in camera* at Kiev and a large number of infringements of Soviet law appear to have been committed. Thus, defence counsel was not granted time for a proper interview and was told by the Judge to base his defence on evidence contained in the prosecutor's files.

A collection of Plyushch's letters has been published in Russian by the Herzen Press in Amsterdam. The first letter was written just after his arrest and the others after his incarceration in the Dnepropetrovsk special hospital, one of the most feared in the Soviet

Union. The letters show a lively, highly intelligent and understanding man for whom the experiences in the hospital were shattering. They show no indication whatsoever of mental illness.

According to information received only last week the 'treatment' received by Plyushch has reduced him to a state of complete nervous collapse so that proper psychiatric treatment under proper humane conditions might really be indicated at the present time.

I am prepared at any time to act as a psychiatrist representing the Plyushch family and would examine Mr Plyushch either at Dnepropetrovsk special hospital, or preferably for all concerned, in a hospital in the UK. Should Mrs Plyushch's application for emigration to the West for her husband, herself and two children be granted, I would be prepared to participate in any therapeutic measures that might be considered necessary to restore him to his previous good health.

G. A. LOW BEER

*Consultant Psychiatrist,
Horton Hospital,
Epsom, UK*

Hungarian visas

Sir,—On behalf of the Presidency of the Hungarian Biochemical Society and also in the name of those who made considerable efforts to ensure the successful organisation of the ninth FEBS Meeting in Budapest in 1974, allow me to express my profound disapproval of the letter entitled, "Entry forbidden" (December 13, 1974) that was written and sent to me as a New Year's present by Ms Peller.

I firmly believe that you are well aware of the fact that the Ninth FEBS Meeting was a success, and it served well not only the exchange of scientific information, but also international scientific cooperation and collaboration. May I quote a part from the letter of Professor H. R. V. Arnstein, Secretary-General of FEBS, addressed to Academician T. Erdey-Gruz, President of the Hungarian Academy of Sciences? "The organisation and scientific standard of the Budapest Meeting were excellent and I have heard many favourable comments. I am sure that the meeting will long be remembered with pleasure by all the biochemists who were fortunate enough to take part and it will have contributed significantly to scientific cooperation in Europe both now and in the future". Similar appre-

ciations were also received from a number of participants from all over the world.

It is incomprehensible to us why such real and true information about the ninth FEBS Congress did not appear in your journal. We think this would have indeed served international scientific relationships well.

Regarding Ms Peller's visa, the facts can be summarised briefly as follows. Ms Peller's request for a visa arrived in Budapest very late. In spite of this it was dealt with and the visa sent to Vienna, just as were a number of other visas of members of the Israeli Biochemical Society. Ms Peller arrived in Vienna a few days before her visa came through and she had not the patience to wait for it; the visa was left at the Hungarian Embassy in Vienna. I would like to mention that more than 2,000 active members from 38 countries participated in this congress and everybody who arranged their visa in due time was able to enter Hungary.

DANIEL BAGDY

*Secretary of the Hungarian
Biochemical Society*

Nutritional research

SIR,—The very real issue which concerns both John Rivers (January 10) and John Yudkin (January 31) in their recent correspondence on the Neuberger report is the balance which must be maintained between applied nutritional research into topics of human and social concern and the more basic type of research usually associated with nutritional biochemistry. There is a risk, however, that the intensity of their criticism might lead the non-nutritionist to believe that the policy makers, particularly those associated with the Medical Research Council (MRC), have shown little concern over this matter. This would be far from true and it is to correct this possible misconception that we are writing this letter.

In 1971 an MRC subcommittee, of which incidentally Professor Neuberger was a member, recommended a change in policy at the Dunn Nutritional Laboratory and this was subsequently approved by the council as follows: "The council recognise the need to lay foundations for more research designed to investigate specific nutritional problems both in the United Kingdom and overseas and they have agreed that in the future research programme of the laboratory there should be a change in emphasis from basic biochemistry towards applied nutritional studies, in particular clinical and epidemiological investigations. Biochemical research will continue in close relation with applied studies".

This policy statement became the basis of our present research pro-

gramme, all of which is related directly to a specific human or clinical problem. These studies have, however, to rely heavily on a firm backing of fundamental research, for in all too many nutritional disorders we lack the basic information to mount really effective applied programmes. Obesity is a good example. We just do not know why individual people lay down different amounts of fat on apparently similar energy intakes and expenditures. At the Dunn, obesity is being investigated on a broad front, using a whole-body calorimeter together with epidemiological studies into the functional significance of different degrees of obesity in terms of morbidity and exercise potential and metabolic studies on the relative economics of different enzyme pathways.

There is considerable interest concerning the role of dietary fibre and this, too, is the subject for a bivalent approach, defining more accurately the metabolic functions of the unavailable carbohydrates and quantifying, by epidemiological and clinical investigations, the practical benefits or disadvantages which might accrue from increasing the fibre content of the diet.

There is also concern about the nutritional status of elderly people, especially those living alone. Recent evidence has suggested the possible existence of sub-clinical riboflavin and vitamin C deficiencies, but in our present state of knowledge we do not know the real significance of the findings. Again, we have an epidemiological investigation under way to reveal the environmental and sociological factors which are causing these abnormalities, linked with laboratory research to define what they mean. Only in this way can we plan effective action should this prove necessary.

Bone disorders are also a problem of the aged and the role of vitamin D in these is likewise under intensive study.

These are just a few of the community-oriented research projects in the UK and overseas in which we are involved and in which we are trying to achieve the same sort of scientific balance.

It is perhaps unfortunate that on first appraisal the Neuberger report does seem to contain more on biochemistry than on 'social' nutrition, but closer scrutiny will show that it quite specifically states that there is a need for more research in this area as well as on other aspects of human and clinical nutrition. The positive point which comes out of the report and the criticisms it has invoked is that nutritional science is very much alive and of importance, not just in an international context but for the health and welfare of people in the UK as well. Nutrition has to be a broadly based science and

it is up to nutritional scientists to make certain their subject is able to develop along balanced lines. This is what the debate should be all about.

R. G. WHITEHEAD

W. P. T. JAMES

*Dunn Nutritional Laboratory,
Cambridge, UK*

SIR,—As a nutritionist who worked for more than 10 years in the Nutrition Division of the Food and Agriculture Organisation of the United Nations, I feel I must take issue with several statements in the Joint ARC/MRC Report on Food and Nutrition Research.

I strongly support those who have already expressed the view that the Report is excessively biased towards the cellular and subcellular level of nutrient activity, and virtually ignores the fact that nutrition is concerned primarily with the food that people eat. The opportunity has been missed for stressing the inseparable interrelationships between agriculture and nutrition, both quantitatively and qualitatively. Thus one question that urgently needs answering is whether this country can increase its food production beyond the present level that meets only about half of our consumption, and in particular how in doing so it can make the most useful contribution to human nutrition? Such a possibility of collaboration between the two Research Councils is not mentioned.

I am one of those nutritionists who have long believed that, however much research still needs to be done in such areas as the effects of nutrients on metabolism, we already know enough to seek to apply our present knowledge to large scale improvements in the health in our own country and in the rest of the world. But to do this, we need a far more aggressive attack on the problem of how to affect people's eating habits than the lukewarm attitude indicated in the report by such statements as: "On the evidence of published literature, the effect of advertising on food consumption patterns appears not to have been studied," or "It may also be desirable to find ways of changing patterns of food consumption when supplies are limited." Do we need another war, or a series of world food crises, to persuade us that this is a problem crying out for research?

In considering training for research in nutrition, the Committee has clearly not surveyed the facts, for it says that it considers that first degrees in nutrition and food science "make relatively little contribution to research potential." This is simply not true. In my work with FAO, I have for example met graduates in nutrition from Queen Elizabeth College carrying out research

in places as far apart as Ottawa and Accra, Kampala and Colombo, Kuala Lumpur and Freetown. Their research was being done at universities, institutes of agriculture and medicine, and government research laboratories, and was in subjects over a wide range, from anti-oxidants and synergists to infant weaning practices and diets. My own experience suggests that a higher proportion of nutrition graduates are actively engaged in food and nutrition research than graduates from the biomedical fields which the Committee has suggested would be more appropriate.

In the section on training for research in nutrition, there is however one useful remark, that the necessary characteristic of the nutrition specialist is that he should have a broad outlook, and that his function is to catalyse and summarise both teaching and research. Were these not the essential features in the department created by John Yudkin when he was appointed as Professor to the first school of nutrition in this country devoted to undergraduate and postgraduate teaching and research at Queen Elizabeth College at the University of London? Professor Yudkin's attitude towards nutrition was reflected in his teaching colleagues, his research students and is now shared by hundreds of nutrition graduates, working in a wide variety of fields, in many countries round the world.

It would be invidious for anyone to criticise the composition of the Committee, possessing as it did such a wealth of knowledge and experience, or to question the esteem of the co-opted members of its Working Parties. Nevertheless it remains true that only three of the fourteen members of the Committee could consider themselves as expert in human nutrition. Even more extraordinarily, neither the Committee nor the forty or more members of the Working Parties had a single representative from either of the two schools which give first degrees in nutrition and which have made many notable contributions to research over the broad field of nutrition.

We in the Polytechnic at Huddersfield do not accept the views implicit in what must come to be known as the Neuberger Report. We are planning to introduce a course for the degree of MSc in Social Nutrition within a few years which will have as one of its major objectives the training of graduates from food science, nutrition, catering studies and dietetics in the social concomitants of nutrition as a preliminary to preparing them for research in this field.

Yours faithfully,

T. B. MORGAN

*The Polytechnic,
Huddersfield, UK*

Early tunnellers

SIR,—When I discussed early British tunnelling projects (Jan. 31, 1975) a printer's imp substituted "early 1880s" for "early 1800s". In fact Trevithick nearly completed his Limehouse-Rotherhithe tunnel under the Thames soon after Waterloo and the Brunels succeeded in the 1840s. This will be found to help the sense which contrasts the preoccupation with tunnelling at the beginning of the 19th century with the switch to bridging towards the end.

ANGELA CROOME

London SE3, UK

Food from allotments

SIR,—With commendable humility, the World Food Conference in Rome called on science and research workers to bridge the gap between the incompatible elements of its action pack (*Nature*, 252, 518; 1974) and I hope I have disentangled the metaphor correctly.

May I, then, suggest a tactic not considered in Rome—one which would pay off within two years, unlike the global meteorological networks, the intensified research, the remote sensing or the testing of new technology in farmer's fields? I merely suggest that everyone who can, especially in the 'have' countries, feeds himself.

It seems plain to me that hunger cannot be alleviated by shifting money about the world, but only by growing more food. Britain survived two wars on the produce of her allotments (over half the island's food was grown on them). If all who have consciences will devote 150 hours a year to a garden, they will do some good, unlike those who walk a few miles on one day of the year to collect money.

I had better explain the word 'allotment' for an international readership. In several countries, municipalities supply, at a nominal rent, small gardens (allotments) to interested citizens: "A plot of 300 square yards" (say 1/40th hectare) "should supply a family of three all year round" (*Encyclopaedia Britannica*, 1951, article Allotment).

There is nothing in this scheme to attract the notice of the Starvation Industry—no intercontinental jet flights, no twenty-thousand-dollar-a-year jobs, no lush research grants. So we common people must demand that land be set aside for allotments in every town. Then each of us can solve a tiny part of the problem. No more can be expected of us.

How big is the problem? I gather we need 45 million extras rations. If, then, 90 million of us—thirty million families—grow half our own food, the immediate problem is solved. With vigorous promotion, there could certainly be 30 million allotments more in the world within two years. It is no dream to pre-

dict 300 million allotments, world-wide, within five years, if we try.

Those who have no land should demand it, where practical, from their local authorities.

F. P. HUGHES

Ontario, Canada

Vial body wanted?

SIR,—It is now more than ever necessary to minimise waste in laboratories, as elsewhere. This seems to make it imperative to devise means, possibly centralised regionally or nationally, for saving and reusing vials for liquid scintillation counting. It seems that, up to the present time, almost all such vials have been discarded and destroyed after being used once only. The numbers of vials so used and destroyed annually in Britain must be very large. In this department alone, the number last year was about 100,000.

Arguments against reusing vials are well known; the main ones are that: (1) the vials may have been irreparably damaged; (2) the vials may be irreversibly contaminated; (3) arrangements for retaining, decontaminating and washing used vials may endanger the environment and particularly, of course, the health of personnel; and (4) such arrangements are uneconomical. These arguments are valid. There are, however, counter-arguments, which seem to have been made unanswerable by the increasingly adverse economic and environmental conditions in which we find ourselves. The essential argument is that the destruction of once-used vials is unacceptably wasteful as the cost of producing them rises and as the materials from which they are made become shorter in supply.

I should like to propose, therefore, that a committee representing vial users, including university, research council and industrial laboratories, be set up to investigate urgently the practicability and economics of the centralised collection, storage and preparation of used vials for reutilisation. There should be little difficulty in collecting the information required for establishing quite rapidly whether this can be done economically on a regional basis. Individual laboratories should find no insuperable difficulties in collecting, storing and despatching used vials safely and efficiently.

If those who agree with this proposal and are prepared in principle to act on it would write to me, the response will be communicated to the research councils and other official organisations in the hope that action will be initiated.

Yours faithfully,

G. V. R. BORN

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news and views

The programming of the visual cortex

by Miranda Robertson

IN the past five years the organisation of the mammalian visual cortex has become the focus of a nature-nurture controversy whose claim to more than usual interest lies in its definition at the level of single neurones and their connections in the brain. The neurological foundations of the debate have been laid very largely by Dr David Hubel and Dr Torsten Wiesel, and the relative absence of dispute over their picture of the neurophysiological basis of vision is almost more surprising than the disagreement over whether, as they think, it is innate, or, as many others insist, it is subject to substantial environmental modification in early life.

The essence of the original description of adult cat cortex (Hubel and Wiesel, *J. Physiol., Lond.*, **160**, 106–154; 1962 and **165**, 559–568; 1963) is thus unchallenged. Cells in the primary visual cortex (area 17) respond to simple line stimuli such as light slits, edges and dark bars. The nature and selectivity of the responses vary with depth from the cortical surface in a way that reflects the specific connectivity of the cells, which Hubel and Wiesel have classified as 'simple', 'complex' and 'hypercomplex'. The functional subdivision of the cortex into layers of simple and more complex cells has since been correlated with the anatomical division into layers of stellate and pyramidal cells (van Essen and Kelly, *Nature*, **241**, 403–405; 1973). At right angles to the layers, the cortex is divided into columns of cells whose responses are tuned to lines of a specific orientation.

In more recent work, Hubel and Wiesel have extended the principle of columnar organisation in work on the distribution of ocular dominance in the monkey, which has revealed alternating sheets of cells fed predominantly by right and by left eyes (*J. Physiol., Lond.*, **195**, 215–243; 1968). They have now gone on to examine more precisely the columnar geometry of orientation specificity and its relationship with ocular dominance. In their three most recent papers they present an arresting picture of the 'functional architecture' of areas 17 and 18 of the monkey cortex, hinting at its implications for the genetic specification of neuronal

organisation and remarking on the influence of environmental factors only for its apparent absence.

The first of the three papers (*J. Comp. Neurol.*, **158**, 267–294; 1974) is concerned with quantitative and morphological definition of the ocular dominance and orientation columns. Hubel and Wiesel now see the columnar organisation as a device for mapping ocular dominance and orientation specificity (and probably other variables, such as colour, which they did not investigate) systematically on to a three-dimensional space whose axes are already engaged with the topographic representation of the visual field (for the surface coordinates) and complexity of processing (for the depth coordinate). From this and their second paper, which is concerned with receptive field size, they have inferred a structure of crystalline regularity, composed of myriad identical columnar machines, each equipped for the complete analysis of a portion of the visual field equivalent in size to the receptive fields of the individual neurones of a column, plus their scatter. They have described the fundamental analytical unit as a hypercolumn—a functional aggregate comprising a left-right pair of ocular dominance columns and a set of orientation columns containing all the specificities in a 180° cycle.

The measurements which led to this inference were made by advancing a microelectrode 20–50 μm at a time tangentially through the cortex, recording the ocular dominance and orientation specificity of each cell encountered and marking the electrode track for later histological examination. Hubel and Wiesel found that ocular dominance alternates regularly at intervals of about 0.25–0.5 mm, and orientation preference changes regularly clockwise or anti-clockwise, with occasional abrupt reversals, completing a cycle within about 0.5–1 mm. Orientation columns seem, like ocular dominance columns, to be sheets rather than cylinders, although the resolution of electrophysiological techniques is so far inadequate either to establish this precisely or to determine whether the changes in orientation preference are discrete or continuous. Nor is the relationship between ocular dominance and

orientation columns clear, although the two systems seem to be independent.

The hypercolumn emerged from a comparison of the sizes of orientation and ocular dominance columns, which differ by an order of magnitude. This struck Hubel and Wiesel as odd until they realised that a right-left ocular dominance pair is about the same width as a complete 180° cycle of orientation preferences: fuse the two and you have a hypercolumn.

The dimensions of the orientation columns have further functional implications. Neuroanatomical studies of cortical cells have shown that the dendritic arborisation of pyramidal cells extends well over the width of an orientation column. This supports the suggestion that the fine tuning of orientation-selective neurones is mediated by lateral inhibition—a postulate for which there is already neuropharmacological evidence (Pettigrew and Daniels, *Science*, **182**, 81–82; 1973; Rose and Blakemore, *Nature*, **249**, 375; 1974).

The second paper is concerned with the uniformity of the hypercolumnar machinery across area 17 of the cortex. The density of the retinal projection to the striate cortex is far from uniform: the density of retinal cells and thus of visual input decreases with eccentricity from the fovea. This is reflected in an increase in the receptive field size of cortical neurones with eccentricity of retinal projection. It is not, however, reflected in any variation in cortical cell density or columnar width: foveal and peripheral information seems to be processed identically.

It is the last of the three papers that contains the basis for contention, for it is here that Hubel and Wiesel describe the extension of the investigations of the preceding two to young monkeys without visual experience. They used four animals. Two whose eyelids were sutured within two days of birth, and one whose eyelids were sutured immediately on delivery by caesarian section, were examined after a few weeks. In one monkey, recordings were made two days after birth.

From data on some 300 cells in all, including 23 in the normal 2-day old, they conclude that the structure they have described for adult cortex is complete and fully functional from birth.

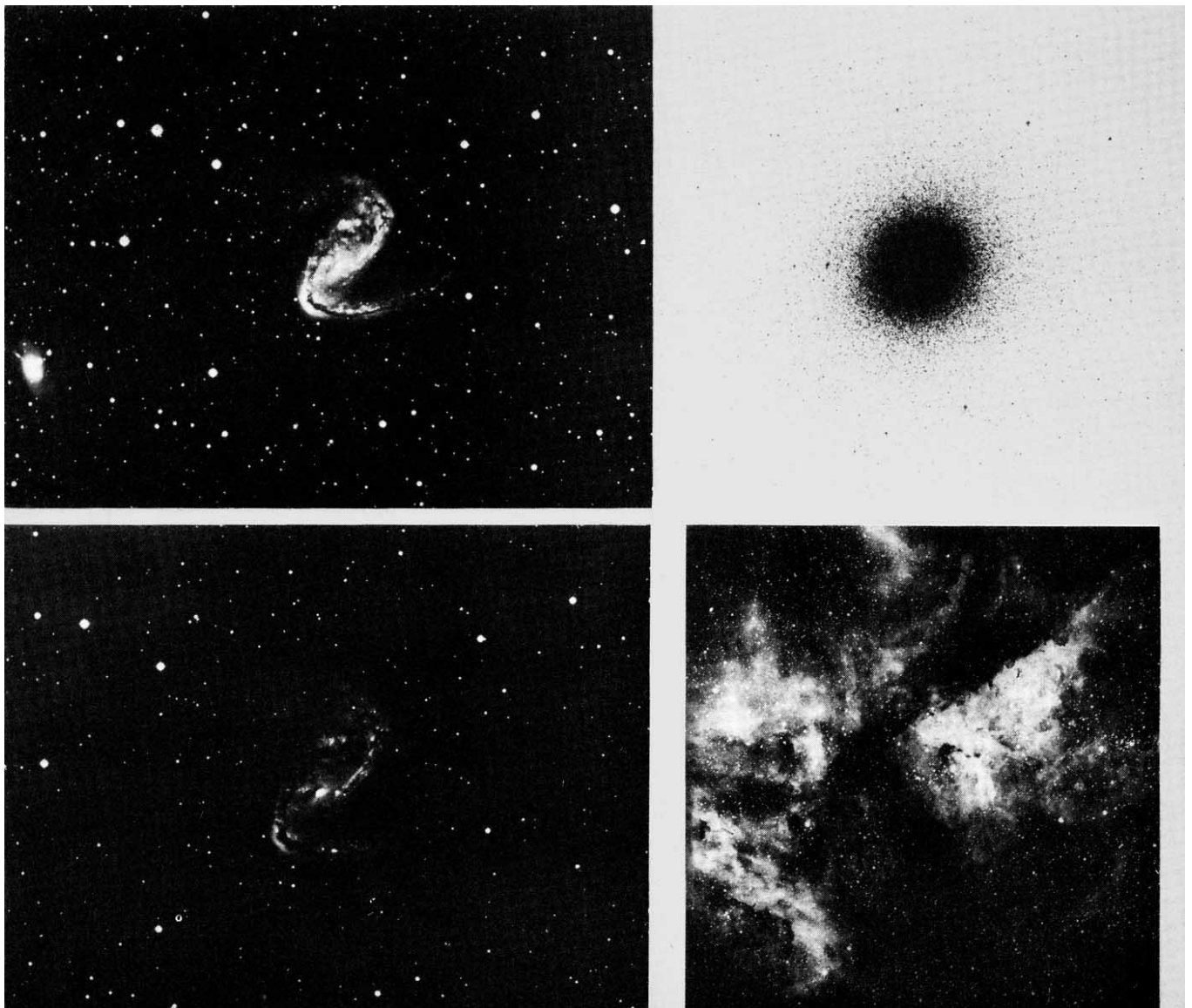
They did detect minor deviations from the normal pattern: a few cells lacked clear orientation specificity, and an abnormally low proportion of neurones responded to binocular stimuli. This is attributed to the early effects of visual deprivation, which is the only environmental factor whose influence they acknowledge. Both ocular dominance and orientation specificity can be disrupted by depriving the cortex of input; but the modification involved represents a non-adaptive deterioration in the existing cortical organisation.

This is precisely the position Hubel and Wiesel adopted on the cat ten years ago, and which has been called into question by the later experiments of Barlow, Blakemore and Pettigrew (Barlow and Pettigrew, *J. Physiol.*,

Lond., **218**, 98–100P; 1971 and Blakemore and van Sluyters, *J. Physiol.*, *Lond.*, **237**, 195–216; 1974), who claim that neither orientation specificity nor ocular dominance is fully determined in newborn kittens, and that both develop in the first weeks of life under the influence of visual input. Hubel and Wiesel now concede, in the light of this and neuroanatomical evidence, that the kitten cortex is immature at birth. Monkey cortex, however, is anatomically mature and, it now seems, neurophysiologically indistinguishable from that of the adult; since its columnar organisation is also in all known respects closely similar to that of the adult cat, Hubel and Wiesel argue that the development of adult properties in cat neurones is a function

of maturation and not of experience.

How is this view of a genetically programmed, structurally uniform, functionally stereotyped repeating crystal of miniature machines to be reconciled with the mass of evidence accumulated over the past five years by (most notably) Blakemore, Pettigrew, and Mitchell, for profound and permanent modification of the orientation specificity and binocular connections of cortical cells by early experience? Numerous experiments on kittens reared in exclusively spotted, vertically striped, or horizontally striped worlds have demonstrated that the majority of cells in the cat visual cortex respond preferentially only to orientations which they have experienced. Blakemore and Pettigrew (Blakemore and van Sluy-



Some of the spectacular photographs already obtained with the 3.9 m Anglo-Australian Telescope at Siding Spring. Left, two exposures of NGC 2442, a barred spiral galaxy with asymmetrical arms and a bright nucleus. Right, two objects in our Galaxy: top, the giant globular cluster 47 Tucanae which is only 14,000 light years away and contains some 10^6 stars; bottom, Eta Carinae, a large hydrogen emission nebula with wide dust trails and narrow lanes ('elephant trunks') where cold interstellar matter is squeezing into the hot gas. Eta Carinae's central star may be a new one, as yet unstable.

ters, *J. Physiol., Lond.*, **237**, 195–216; 1974 and Pettigrew, *ibid.*, 49–74) propose that immature cells can respond to a wide range of orientations which is narrowed as a consequence of experience, probably through the strengthening of inhibitory intracortical connections which 'tune' the response curves of the complex and hypercomplex cells. Blakemore and van Sluylers have gone further, and suggest that orientation preference is innately biased in the simple cells, which transmit the bias to the immature complex and hypercomplex cells of a given column and thus provide an innate substrate for the regular repetition observed by Hubel and Wiesel. But in their visually deprived monkeys, Hubel and Wiesel found representatives not only of simple cells, but of complex and hypercomplex cells with adult properties.

Blakemore and van Sluylers insist that visual experience actually modifies cortical connections, and does not simply cause selective deterioration: there are no silent areas. For example, monocular suturing of kittens' eyes reversed during the plastic period of development (up to 14 weeks) causes a reversal of the cortical ocular dominance pattern.

For aspects of the nature–nurture conflict on which both factions have experimental evidence, the best hope of a resolution in favour of plasticity lies in the experimental procedures used by Hubel and Wiesel. There was at one time a suggestion that Hubel and Wiesel had mistakenly recorded as orientation detectors cells which were in fact merely movement detectors, which are relatively crude entities characteristic of immature cortex. They have now, however, introduced controls which go some way towards answering these criticisms. The other question which has been raised is the completeness of the early visual deprivation. Evidence has recently been presented that cortical modification can take place within as little as an hour's experience (Pettigrew, Olson and Barlow, *Science*, **180**, 1202–1203; 1973). The speed and effectiveness of the suturing of the eyelids of newborn monkeys (whose cortex everyone agrees is anatomically mature) thus becomes critical.

Otherwise, the only way of reconciling the conflicting observations is by the intuitively unappealing compromise of a cortex whose elaborate and fully functional innate organisation can be substantially respecified as a result of experience. This is horribly unpar-simonious because if cortical plasticity does not take the onus of functional adaptation off the genome it is hard to see its evolutionary advantage in a visual world which as far as we

know has been fairly constant.

The question of the evolutionary significance of cortical plasticity has been specifically addressed by Blakemore and van Sluylers, who believe that its crucial function may be the matching of orientation specificity for the inputs of both eyes to the binocular cells of the cortex. Binocular cells as defined by Barlow *et al.* (*J. Physiol., Lond.*, **193**, 327–342; 1967) have an essential property that has not been examined by Hubel and Wiesel in their binocularly driven cells. They represent the neurological substrate of depth perception and are acutely sensitive to disparities in the position of the image on the two retinæ, which vary as a function of distance. Clearly the input to a binocular cell must be tuned to precisely the same orientation for both eyes. Blakemore and van Sluylers concur with Hubel and Wiesel in finding a few (20%) cells with binocular connections in the absence of visual experience. They have shown, however,

that without patterned stimulation the orientation preference of the two inputs is poorly matched. Hubel and Wiesel have not reported on the matching of inputs to the binocular cells in visually inexperienced monkeys.

Thus, much of the contention devolves upon how much is still not known about cortical function. It is now agreed that at least a part of the structural basis for visual function is laid down at birth; the question is whether it is then modified and refined by experience. This question cannot even be definitively posed while it is still unclear how much is not known about the visual cortex. It has been estimated that neurophysiological techniques sample something like 50 cells in a population of about 2,500 encountered by an electrode in the course of a single penetration, and there is no guarantee whatever that the sample is representative. It is thus surprising that the lines of dispute can be drawn so clearly on a basis of accepted data.

Palaeomagnetic field matters

from Peter J. Smith

ONE of the most fascinating phenomena in the history of the earth sciences is the way in which palaeomagnetic research and the arguments about continental drift developed in parallel for over 40 years, coexisting within the scientific world but never really meeting. Possible reasons for this intellectual failure are not difficult to find in retrospect. For one thing, as a reading of Koenigsberger's classic reviews shows (*Terr. Magn. atmos. Elect.*, **43**, 119 and 299; 1938), interest in the magnetism of rocks (going back well into the eighteenth century) was largely a concern for what would now be termed rock magnetism rather than palaeomagnetism—the solid state physics and mineralogy of magnetic grains as opposed to any relevance the magnetism might have to the history of the Earth. As such, it was a subject to be pursued by physicists rather than geologists and thus liable to fall victim to the growing separation of the sciences and increasingly difficult communication. Wegener's hope that his *Die Entstehung der Kontinente und Ozeane* (1915) would re-establish the connection between geophysics on the one hand and geology and geography on the other was not to be fulfilled for many a year.

The second, perhaps more important, reason for the early failure to connect palaeomagnetism with the continental drift debate was that the few genuine palaeomagnetists about had something quite different in mind. As late as 1949, for example, Graham (*J. geophys. Res.*, **54**, 131; 1949) could head his objectives

with the claim that "if this pattern of primary magnetisation [in sedimentary rocks] could be shown to be permanent . . . it should be possible then to determine how the direction of the Earth's magnetic field has varied during geological time". The previous year, Johnson *et al.* (*Terr. Magn. atmos. Elect.*, **53**, 349; 1948) had made their primary aim even more explicit: "In order to determine the origin and nature of the Earth's magnetic field and to test the various hypotheses which have been advanced to explain the field, it is desirable to determine the history of this field throughout geological time and to investigate more carefully its spatial variations, both inside and outside the Earth's surface." Their own work on Pacific sediments and the glacial clays of New England was one of the first important steps in this direction.

When the palaeomagnetic method and the hypothesis of drifting continents did finally come together in the late 1950s the mixture was explosive. The combination also changed the palaeomagnetic ground rules as they would have been understood by Johnson and his colleagues. For one thing, the growing evidence for drift made it clear to the pre-revolution palaeomagnetists that they were to be thwarted in one of their primary tasks—that of using the magnetism of rocks to test directly whether or not the geomagnetic field had always been essentially dipolar. From now on the dipole hypothesis would become a

necessary assumption (not without considerable justice) rather than an idea to be confirmed or refuted. Perhaps more significantly, the aims of palaeomagnetism were expanded to cover not just the relatively narrow geophysical problem of tracing the geomagnetic field through time but also the much wider geological problem of delineating the movements of land masses. Or to put it another way, the wider geological community had begun to discover the Earth's magnetic field. But in bringing the larger and smaller communities together it was inevitable that henceforward the geological aspects of the subject should dominate the geophysical ones.

Nevertheless, fundamental work on the origin and long-term behaviour of the geomagnetic field continued throughout the earth science revolution, albeit on a relatively small scale; and some of it (most notably the construction of the geomagnetic polarity-time scale) even found important geological application in its own right. In recent times, however, there has been a noticeable upsurge of interest in the study of the palaeomagnetic field for its own sake, almost 20 reports having been published in the last six months alone. And it is apparent from the diversity of subjects covered that the detailed delineation of the history of the Earth's magnetic field is still far from complete; even the comfortably familiar is not beyond question.

The article by Verosub on page 707 of this issue of *Nature* offers a good case in point. Since the pioneering work of Elsasser (*Phys. Rev.*, **69**, 106; 1946 and **70**, 202; 1946) in the mid-1940s, the self-reversing dynamo has come to be accepted as the only viable source of the main dipole field. There are problems in establishing the validity of the dynamo model because, although mechanical analogues can reproduce field reversals and field intensity fluctuations, they are a poor substitute for the complex motions thought to occur in the Earth's core; and the motions themselves have not so far proved amenable to satisfactory mathematical treatment. Nevertheless, the self-reversing dynamo has reigned supreme if only because every other idea has been ruled out as impossible. Or so we thought. Verosub, however, offers a delightfully simple (in principle) alternative involving two opposing fields whose magnitudes vary with time. This model poses its own problems, of course—most notably the need to find the origin of the second field (the first presumably being produced by a non-self-reversing dynamo in the fluid outer core). But by the same token, it also brings the whole question of field origin back within the scope of non-magnetohydrodynamic man.

In support of his two-component field source hypothesis Verosub quotes Wilson's (*Geophys. J.*, **28**, 295; 1972) discovery that there are significant differences between the time-averaged mean pole positions of normal and reversed populations—differences which Verosub believes might indicate the dominance of a distinct field source in each polarity state. Using Upper Tertiary and Quaternary palaeomagnetic data from

the Soviet Union, Wilson showed that whereas both normal and reversed poles tend to lie on the far side of the present geographical pole from the observing site, the 'far-sidedness' in the reversed case is significantly greater than in the normal. Earlier, Wilson (*Geophys. J.*, **19**, 417; 1970) has interpreted far-sided poles in terms of an axial dipole displaced a small distance northwards from the centre of the Earth; and this is a theme to which

Time to ecological equilibrium

Responses from Leigh Van Valen, T. R. E. Southwood and Donald Strong

STRONG (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2766; 1974) has pointed out flaws in the correlation shown by Southwood (*J. Anim. Ecol.*, **30**, 1; 1961) between subfossil records of trees and the associated insect species richness. He showed that Southwood's correlation was confusing by demonstrating a correlation between the present range of British trees and the number of associated insect species. He also estimated that the time required for tree genera introduced into Britain to become saturated with herbivores was only a few decades or hundreds of years—the evidence for this being that the regression of herbivores on the distribution of non-native trees does not differ significantly from that of native genera. Peter D. Moore discussed his findings in *Nature* (**252**, 14; 1974).

● Leigh Van Valen now writes that Strong's last conclusion is unjustified because "analysis of data from Strong's Fig. 3 shows that the slope of regression for introduced genera not only does not differ significantly from that for native genera, it also does not differ significantly from either 0 or 1, whether or not two ambiguous genera (apple and lime) are included. Therefore the conclusion, while quite possibly true, is not supported by the data."

● Peter Moore, who expressed in his article in *Nature* reservations about Strong's evidence against an increase in species richness over geological time, now adds, "I agree that one cannot justify a rapid attainment of saturation by insect species on the basis of Strong's data. On the contrary, one might predict that if any non-native tree species in Britain were to extend its range significantly then a rise in associated insect species might be expected. The question of changes in richness during geological time remains unsettled."

● T. R. E. Southwood emphasises

that although he is responsible for the correlation between geological time and species richness, in his original paper he also pointed out the relationship between the present range of trees and their associated species richness (see *Proc. Hawaiian ent. Soc.*, **17**, 299; 1960; *XI Int. Kongress fur Ent.*, **1**, 651–655; 1961 and *Nature*, **253**, 313; 1975).

● Donald Strong replies: "Van Valen is wrong. Given the correlation coefficient values characteristic of host plant–insect species richness relationships, one does not expect high significance values for comparisons of few points; my conclusions are not contingent upon the significance of the correlation of introduced points. Since the original publication, however, I have been supplied range data on two additional introduced hosts, walnut and spruce (through the great courtesy of Dr Perring's unit at Monk's Wood). With these included, the correlation coefficient of introduced taxa is significantly greater than 0 ($r=0.635$; $0.05 < P < 0.1$). These data will be included in a forthcoming paper dealing with other pest species over these same hosts. This new paper will demonstrate rapid species saturation as does the first. As well, I have found rapid saturation in a totally different plant–insect system (*Science*, **185**, 1064–1066; 1974).

"Southwood's comment is correct, and Moore is confused, as reference to my original paper will verify. One of my main theses was that pest species richness will vary as host range varies, quickly, be the species introduced or native. My alternative to the non-asymptotic model was clearly stated to be an asymptotic one."

"Please note also that there is an error in my original paper; in Fig. 3 'common maple' sits not to the left of the regression line as I have plotted it, but virtually on top of it. This correction reinforces my conclusions."

he and McElhinny (*Geophys. J.*, **39**, 571; 1974) have now returned. An up-to-date analysis of 291 worldwide late Tertiary and Quaternary palaeomagnetic pole positions confirms the phenomenon of far-sidedness and also suggests that poles tend to lie to the right of the geographical pole when seen from the observing site. The revised figure for the average northward offset of the dipole for the past 25 million years is 325 ± 57 km (standard error), but there are now enough data available for them to be divided into smaller sets. The dipole offsets for the periods 25–7, 7–2 and 2–0 million years are 555 km, 316 km and 143 km, respectively, showing that the axial dipole has been moving gradually towards the geocentre.

On this occasion, Wilson and McElhinny did not consider the normal and reversed data separately, so it is not yet possible to say whether the time-averaged differential offset indicated by the Soviet results is valid on a worldwide basis or not. Looking at a shorter time scale, however, the whole question of polarity states and reversals continues to exercise the apparatus of the mind and the laboratory in a variety of ways. Aldridge and Jacobs (*J. geophys. Res.*, **79**, 4944; 1974), for example, have constructed mortality curves for each normal and reversed phase of the Earth's magnetic field over the past 45 million years and find that they differ from a simple exponential distribution for the lifetime of the phases. It is possible, of course, that the distribution should not be exponential anyway. But Aldridge and Jacobs consider it more likely that the observed departures from an exponential form arise from short polarity events that have not yet been detected—a conclusion similar to that reached, albeit in different ways, by several others beginning with Cox (*J. geophys. Res.*, **73**, 3247; 1968).

The ratio of the number of real polarity phases to the number of those actually observed so far is apparently about 2. This indicates an appreciable number of reversals remaining to be discovered, and may go a long way towards explaining the prevailing confusion over the identification of short polarity events. As Noël and Tarling point out on page 705 of this issue of *Nature*, for example, the so-called Laschamp event has been detected in at least 12 localities at reported ages ranging from 7,000 to 17,000 years. But are all the claims for the recognition of the Laschamp valid, or are there really two or more events involved here? And what is an 'event' anyway? Some years ago a polarity event was defined as a short period ($<10^5$ years) of opposing polarity within a much longer polarity epoch. But McElhinny (*Palaeomagnetism and Plate*

Tectonics; Cambridge University Press, 1973) has suggested that brief events such as the Laschamp may not be true reversals at all but only 'excursions' of the geomagnetic field—relatively short-term deviations of the palaeomagnetic pole beyond that reasonably attributable to normal secular variation but something less than a full 180° reversal. Indeed, some workers, such as Freed and Healy (*Earth planet. Sci. Lett.*, **24**, 99; 1974), have already taken to referring to the 'Laschamp excursion'.

But how do field excursions arise? (They are known to occur, irrespective of whether or not the Laschamp event is one of them.) Possible causes are a tipping of the dipole towards the equator or a reduction in dipole strength which would allow the non-dipole field components to predominate. The latter is perhaps the more likely; and in this connection it may be significant that Aldridge and Jacobs mention dipole strength as possibilities for their missing events, that Cande and Labreque (*Nature*, **247**, 26; 1974) offer field intensity fluctuations as a possible explanation of the small scale magnetic lineations recorded over the North Pacific floor by deep tow magnetometers, and that Greenewalt and Taylor (*J. Geophys. Res.*, **79**, 4401; 1974) claim that low magnetisation blocks detected in the mid-Atlantic ridge "could represent actual periods when oceanic crust was generated during a time of significantly reduced main field strength". In short, there are clear suggestions that dipole fluctuations may account for several phenomena which are otherwise difficult to explain.

Which brings matters to one of the most intractable of all palaeomagnetic problems—that of how to determine the strength of the ancient geomagnetic field and plot its variation, if any, with time. There are two main difficulties here. The first is that although a palaeomagnetic direction may be obtained from a rock just as long as a measurable amount of the primary magnetisation remains, a palaeomagnetic field intensity may generally only be determined if the proportion of primary magnetisation remaining is known. It seldom is, although ways are available of mitigating the problem in certain cases. The second difficulty is that the obvious ways of obtaining a palaeofield from a rock containing thermoremanent magnetisation require that the rock be heated above its Curie point, a process likely to alter the magnetic minerals either physically or chemically. Clearly it would be desirable to have a method of determining ancient field intensities which could either be applied at room temperature or, if heating is necessary, would enable any alteration in the rock to be avoided or estimated. Curiously, three possible



A hundred years ago

THE fitting of the Arctic ships *Alert* and *Discovery* is making rapid progress at Portsmouth, in the hands of the dockyard shipwrights, who are working extra hours, in order that they may be rigged and out of their hands by the 12th of April. The sledges have all been made, and the tents are in progress. Meanwhile the officers are pursuing their special studies. We understand that Commander Markham, and Lieutenants Archer, Giffard and Fulford are going through a course of instruction in magnetism. Lieutenants Parr and May are to be initiated into some special astronomical work, and two other lieutenants will receive charge of the pendulum observations. The work connected with spectrum analysis will also be provided for, and one or more of the officers will take up photography. The ships will be commissioned in the middle of April, and will sail early in June.
from *Nature*, **11**, 355; March 4, 1875

candidates have appeared almost simultaneously, all of them involving anhysteretic remanent magnetisation (ARM). The most satisfactory appears to be Shaw's (*Geophys. J.*, **39**, 133; 1974), a method which does not eliminate heating but which permits selection of a coercive force region within which the heating has not changed the magnetic properties. Banerjee's method (*Earth planet. Sci. Lett.*, **23**, 177; 1974) requires a single heating but is claimed to be preferable to those methods requiring repeated heatings; and the technique described by Stephenson and Collinson (*Earth planet. Sci. Lett.*, **23**, 220, 1974) involves no direct heating but requires the assumption that parameters relating to some rocks are also valid for others. No method is completely problem-free.

Finally, it must be said that this discussion of recent work on the palaeomagnetic field is far from exhaustive. During the past few months Georgi (*Geophys. J.*, **39**, 71; 1974) has presented a spherical harmonic analysis of palaeomagnetic inclination data, Baag and Helsley (*J. geophys. Res.*, **79**, 4918 and 4923; 1974) have constructed a new geomagnetic secular variation model and carried out a shape analysis of palaeosecular variation data, Creer (*Earth planet. Sci. Lett.*, **23**, 34; 1974) has determined geomagnetic variation recorded in a Black Sea sediment core 7,000–25,000 years old . . . and so on. Statistical anomaly or new commitment?

Solar influences on the weather

from R. H. Olson

A symposium on solar-weather relationships sponsored jointly by the American Meteorological Society and the Solar Physics Branch of the American Astronomical Society was held in Denver on January 23.

THE solar-weather symposium was designed to focus the attention of both the meteorological and astronomical communities on this perplexing problem. Primary emphasis was not on reporting of new empirical findings, but on physical concepts. For example, J. Toomre of the University of Colorado compared the problems of describing the convective activity on the Earth and on the Sun. In spite of the difference of many orders of magnitude in the Rayleigh and Prandtl numbers on the two spheres, some similarities exist. A mathematical technique borrowed from meteorology has been found useful by solar physicists in describing solar convection. It is based on the anelastic equations, which assume a quasi-compressible fluid. In similar vein, P. Gilman of the National Center for Atmospheric Research (NCAR) compared the horizontal circulation patterns of the Earth and Sun. He pointed out the great importance of eddy components in driving both circulations. The importance of convection on the Sun as opposed to solenoidal fields on the Earth was mentioned in describing the energy sources for the circulation. Perhaps the greatest unsolved problem in the solar circulation is to explain the increase in rotation speed toward the equator (that is, the equatorial acceleration).

Empirical findings were not entirely neglected. J. Eddy of NCAR gave a historical review of the field. He reminded the audience of a period of almost complete absence of sunspots which occurred from about 1645 to 1715. This 'Maunder minimum' was discovered in the early years of the century, but has been largely forgotten. It is an intriguing thought that this greatest known anomaly in the behaviour of the Sun comes in the middle of the greatest climatological event in recent centuries, namely the 'Little Ice Age', a period of abnormally low temperatures and precipitation.

Other statistical relationships were pointed out by J. King of the Appleton Laboratory (Slough). He gave examples of 11-year and 22-year periodicities in weather phenomena. But R. Shapiro of Air Force Cambridge Research

Laboratories pointed out that such apparent cycles are difficult to prove.

Getting around to the physical side of solar-weather relationships, the cupboard is still fairly bare. There are some interesting possibilities, however. R. Dickinson of NCAR suggested that the only physical process known to have a large solar cycle modulation and reaching the troposphere is Galactic cosmic ray ionisation. This ionisation may nucleate sulphuric acid aerosol, which in turn could provide efficient cloud condensation nuclei, thus modulating cirrus cloud cover at high altitudes and latitudes. He mentioned recent findings that solar flares cause large and sudden increases in NO_x species in the upper atmosphere, which can lead to large scale destruction of ozone.

A. Dessler of Rice University summed up possible mechanisms which might bear investigation. One which has not been widely discussed before is the leakage of electric current from the auroral region into the lower atmosphere. He also developed the theme touched on again and again by Eddy and other speakers, namely that our knowledge of the possible variations in the so-called solar constant is abysmally poor. Variations of anywhere from 0.1% to 0.5% or more could easily go undetected with our present observing capabilities. Even if the lower figure is examined, it is obvious that the energy available from possible variations in the solar constant is 1,000 times the energy available from the magnetosphere, as far as influencing the atmosphere goes.

The problem of the lack of knowledge of the solar constant also provided perhaps the closest thing to a clash of opinions. D. Williams of the National Oceanic and Atmospheric Administration suggested that in view of the gross uncertainties in this parameter it was pointless to pursue solar-weather studies until this area is cleared up. W. Roberts of the University of Colorado retorted sharply that he was not willing to wait five years or longer to go on looking into the important problem of solar-weather relationships. Perhaps some progress in measuring the solar constant with high precision is in the offing. D. Heath of NASA Goddard mentioned that Nimbus satellites to be launched in 1975 and 1978 are scheduled to repeat from space the surface-based measurements made by Drummond and others of the solar constant. Other satellites will attempt to improve the global monitoring of climatic elements, such as ocean environment, atmospheric heat budget, and trace elements. Global maps of precipitation are being made now by satellite, and will soon be available for use.

The great importance of the Soviet efforts in solar-weather studies was at least touched upon, although one had the feeling that only the tip of this particular iceberg was showing. V. Mikhnevich of the Applied Geophysical Institute, Moscow, reported on attempts to derive mathematical-physical models to explain the propagation of disturbances, caused by solar activity, in the upper atmosphere down to the lower atmosphere. G. Gromova of the Hydrometeorological Centre, Moscow, reported on a study of the kinetic energy budget of the atmosphere, averaged over latitudinal zones. An increase in kinetic energy three days after magnetic storms was contrasted with reduced kinetic energy following geomagnetically quiet times.

King suggested that atmospheric modelling is needed to set the framework for solar-weather relationships. J. Wallace of the University of Washington and NCAR replied that before useful models could be built a definitive pattern of observed relationships is needed. C. Leith of NCAR remarked that current models of short-term atmospheric changes have serious shortcomings in their ability to explain atmospheric changes. Thus there is ample room for the models to include such additional inputs as solar influences if any can be shown to exist.

The Earth as a radio source

from A. P. Willmore

AMONG the planets, we are accustomed to think of only Jupiter as a powerful radio source, but results obtained recently with the satellites Imp 6 and Imp 8 in interplanetary space show that the peak radio emission from the Earth exceeds that from Jupiter by nearly two orders. The experiments, described by Gurnett (*J. Geophys. Res.*, **79**, 4227; 1974), were designed to study plasma waves propagating in the magnetosphere and interplanetary space, the geocentric distance of Imp 6 ranging from 6,613 to over 200,000 km, while that of Imp 8 ranged from 150,000 km to 300,000 km. The spacecraft were each equipped with long dipole aerials of lengths approximately 50–100 m and with three mutually orthogonal loop aerials, so that both the electric and the magnetic field components of the waves could be measured. The aerials were coupled to sensitive wide-band amplifiers whose output was analysed by multichannel spectrum analysers covering the spectral range 36 Hz to 178 kHz, extended up to 2.0 MHz by a tunable receiver in the case of Imp 8.

At distances of several Earth radii, Imp 6 detected sporadic bursts of noise

in its upper frequency channels, above about 30 kHz. The duration of the bursts ranged from 0.5 to several hours, with intervening intervals of as much as 24 hours during which the signals might be undetectable. The first problem was to identify the type of wave being detected. On comparing the results from the two sets of aerials, it was found that the electric and magnetic field intensities were linearly related, with a constant of proportionality which identified the waves as ordinary electromagnetic waves. The frequency spectrum of the bursts was determined with Imp 8 and shown to be remarkably narrow, most of the

radio wave energy being contained between 60 and 300 kHz.

When Imp 8 was at a distance of about 200,000 km from the Earth, the measured electric field signals were found to be strongly spin modulated, showing that the waves originated from an approximately point source, since had their distribution been isotropic the signal would not have depended on the orientation of the spacecraft. In fact the source was shown to have an angular diameter of less than 12° and to be in the direction of the Earth. At this distance, the diameter of the Earth subtends 4° , so though the Earth was clearly the source, its precise location

on the Earth could not be determined. On the other hand, a source relatively low in the atmosphere, rather than in the magnetosphere, is suggested, a supposition which is rather strengthened by the observation that, statistically, the intensity of the bursts falls off in an inverse square law fashion with distance from the Earth, at least at considerable distances (more than 4 Earth radii).

More precise information on the location of the source, however, was obtained from the intensity variations at various points in the magnetosphere. The waves were not observed whenever the spacecraft were inside the boundary surface known as the plasmapause.

LONGO and Penhoet reported¹ that a rat glioma releases, among other molecules, a protein which shares some immunological, chemical and biological properties with the mouse salivary nerve growth factor (NGF). A correspondent writing in these columns² about their work raised the question of a possible new role for the glial cell. Though this question is legitimate on theoretical grounds I wish to point out other findings which would not support the hypothesis.

NGF is present in large quantities in snake venom and male mouse submaxillary glands, but these are not the only sources of NGF. This protein molecule is also released from some mouse sarcomas³ and is produced by granuloma⁴ and embryonic tissues⁵. Recently a sensitive immunoassay was used to show that two neoplastic cell lines, L and 3T3, derived respectively from mouse C3H subcutaneous and adipose tissues and from simian virus-40 transformed A31, likewise produce a biologically active NGF which is immunologically similar if not identical to mouse submaxillary gland NGF⁶. Since in the experiments by Longo and Penhoet the NGF-like protein was isolated from solid rat tumours which were obviously contaminated with fibroblasts and other host cells, one cannot decide whether the NGF was of glial or fibroblastic derivation, and one wonders why the investigators did not extract the NGF protein from a pure glial cell line rather than from transplanted tumours.

In favour of an NGF-releasing role of glial cells the correspondent also quotes some recent experiments by Swedish investigators who reported that NGF injected intracerebrally enhances regenerative processes in noradrenergic nerve cells in the CNS⁷. Since glial cells outnumber nerve cells in the CNS

A new role for the glial cell?

a reply from Rita Levi-Montalcini

in the ratio of 10:1, these findings seem to the correspondent to suggest that this astronomically large cell population may provide the NGF which could not readily enter the brain from the bloodstream. It should however be remembered that the number of noradrenergic nerve cells in the CNS is of the order of a few thousand (the locus coeruleus, by far the largest noradrenergic nucleus in vertebrate brains, contains about 1,400 noradrenergic neurones⁸) while the whole neuronal population in the mammalian CNS is in the range 10^8 – 10^{10} according to the size and phylogenetic position of brains. Hence only one out of a million nerve cells in the CNS would be receptive to NGF. An NGF-releasing role of glial cells would therefore benefit only an exceedingly small nerve cell population.

The situation in the peripheral nervous system is not much better. Regeneration of damaged axons occurs in peripheral nerves, irrespective of the receptivity of their cells of origin to NGF. Somatic motor and sensory differentiated neurones do not in fact respond to NGF and yet their axons are tightly wrapped by glial cells. Furthermore sympathetic neurones show a maximal NGF response at an early stage of differentiation when only a few glial and other satellite cells are present in the ganglia and are very loosely scattered among, but not adherent to post-ganglionic axons.

The possibility that glial cells surrounding other nerve cell types might contain hitherto undiscovered nerve growth factors cannot be discarded. But before considering this

it is highly desirable to obtain much more convincing evidence for the release of NGF by cells unequivocally identified as glial cells.

The correspondent suggests using immunofluorescence histochemistry to see whether NGF can be found in glial cells which are "wrapped around" their target neurones. But glial cells are not wrapped around noradrenergic neurones in the vertebrate CNS. The locus coeruleus consists of a small population of densely packed nerve cells in reciprocal contact with each other. Glial cells are not seen around individual neurones. Only a few loosely scattered, small non-neuronal cells are found intermingled with nerve cells of this nucleus, and there is no way of deciding whether these are glial cells or other cell types. Since some fibroblastic lines release NGF (refs 4–6) a positive fluorescence reaction would still not prove that glial cells release NGF. Even if they do, the NGF is probably present in the cells that produce it in such small quantities as to be undetectable by this technique.

While therefore there is no *a priori* reason to object to an "NGF-releasing role" of glial cells in the same way as this property has already been proved for a number of other cell lines, the hypothesis submitted by the correspondent that this role would explain the intimate relationship between glial cells and neurones is considerably weakened by the above considerations.

¹ Longo, A. M., and Penhoet, E. E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2347–2349 (1974).

² *Nature*, **251**, 100–101 (1974).

³ Levi-Montalcini, R., and Hamburger, V., *J. exp. Zool.*, **116**, 321–362 (1951).

⁴ Levi-Montalcini, R., and Angeletti, P. U., in *Proc. 4th Int. Neurochem. Symp.* (edit. by Kety, S. S., and Elkes, J.), 362–376 (Pergamon Press, New York).

⁵ Bueker, E. D., Schenkein, I., and Bane, J. L., *Cancer Res.*, **20**, 1220–1228 (1960).

⁶ Oger, J., Arnason, B. G. W., Pantazis, N., Lechrich, J., and Young, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1554–1558 (1974).

⁷ Björklund, A., and Stenevi, U., *Science*, **175**, 1251–1253 (1972).

⁸ Descarries, L., and Saucier, G., *Brain Res.*, **37**, 310–316 (1972).

This divides the charged particle plasma which corotates with the Earth (in fact the upward extension of the ionosphere) from that which is fixed in an Earth-Sun coordinate system due to the existence of the solar wind. That the waves were not observed within the plasmopause is quite natural, since the ambient density of charged particles is too high for the propagation of electromagnetic waves of low frequencies. The fact, however, that a shadow zone could be traced in the magnetosphere out to distances of 15 Earth radii, showed that the waves must originate in the auroral zones of the atmosphere. Finally, correlation of the observation of particular bursts of radio noise with observations of the auroral regions by the USAF Dapp satellite, which obtains a picture of the auroral oval, showed the electromagnetic radiation to originate in discrete auroral arcs, generally in the evening portion of the auroral zones. Evidently the waves are generated by the intense fluxes of energetic electrons which produce the auroral arcs.

The production process must be efficient, since the energy associated with the electrons is typically 10^{11} W, whereas that in the radio bursts is 10^9 W. Certainly some cooperative mechanism or plasma instability is required, which is true also in the case of the radio emission from Jupiter. There are other similarities with Jupiter; among others large fluxes of energetic electrons moving along the magnetic field lines are involved in each case, and the frequency of emission also appears to be similar to the electron cyclotron frequency in the emitting region. Gurnett concludes that the types of instability proposed to account for Jovian emissions may also account for the terrestrial radiation.

Catecholamine receptors detected

from Leslie L. Iversen

THE current vogue for labelling receptor sites has resulted in spectacular progress towards identifying sites for various peptide hormones, neurotransmitters such as acetylcholine and glycine, and drugs, including morphine (see Dismukes, *Nature*, **252**, 442; 1974). Catecholamine receptors, however, have proved more elusive. Various attempts to identify β -adrenoceptors in tissues such as liver, heart or spleen by measuring the binding of labelled catecholamines or their antagonists to intact cells or to microsomal fractions failed to demonstrate a binding process with the expected properties. Cuatrecasas *et al.* (*Nature*, **247**, 92) reviewed their own experience in this field at the

Microwave spectra of molecular ions

from our Chemical Physics Correspondent

MICROWAVE or rotational spectra of neutral molecules are straightforward to study, but the difficulties multiply for short-lived molecular ions in the gas phase. This is unfortunate since interstellar conditions, with a comparative absence of collisions, are much more favourable to long life for such species. Indeed the structure HCO^+ has been tentatively suggested for "X-ogen".

Further interest arises since Bunker (*Chem. Phys. Lett.*, **27**, 322; 1974) has pointed out that species such as HD^+ and $^{14}\text{N}^{15}\text{N}^+$ should have quite strong microwave spectra. The corresponding neutral molecules have zero dipole moment, unless extremely small effects are considered, and consequently they have no detectable microwave spectrum. For charged species the dipole moment is no longer independent of the origin of the coordinates to which it is referred. Although if this origin is at the centre of the ion the dipole moment will be equally small, for discussing microwave spectral intensity, as Bunker shows, the appropriate origin is the centre of mass so that species with different isotopes may have quite strong spectra.

Similarly they have significant infrared absorption intensity. In HD^+ the calculations suggest a dipole of 2.9×10^{-30} C m (0.87 D) and a vibrational transition moment of 2.8×10^{-31} C m (0.086 D).

Alongside this theoretical feature one must place the experimental success in the laboratory of Dixon and Woods (*Phys. Rev. Lett.*, **34**, 61; 1975) in the detection of a spectrum due to CO^+ . A pair of lines at 117 692.55 and 118 101.99 MHz due to $J\ 1/2-1/2$ and $1/2-3/2$ respectively were observed. Special techniques with a sample length of 3.5 m included the discharge to form the ions and a signal averager to enhance the low signal-to-noise ratio through repetitive scanning. Because of the unpaired electron the spectrum is sensitive to magnetic fields and the detection of both lines enables the spin-rotation constant γ_0 to be evaluated as 272.96 MHz and the rotational constant B_0 as 58 983.13 MHz. Although moderately accurate values were previously known from optical spectroscopy, these more precise values should be an excellent guide to radioastronomers who wish to seek for CO^+ .

beginning of 1974 and persuasively demonstrated that the binding of ^3H -noradrenaline to such preparations detected a site that recognises primarily the catechol function of the molecule. This site, unlike the β -adrenoceptor, does not distinguish between the active and inactive stereoisomers of the catecholamines or their antagonists and may be a form of the enzyme catechol-O-methyl transferase.

A great deal of advice and criticism followed during 1974 as to how the job should be done, and it is perhaps not surprising that by the end of the year success should finally have been attained in the search for a method of labelling catecholamine receptors. Indeed, three groups have now reported binding assays which exhibit the characteristics predicted for β -adrenoceptors. Their success in the face of many previous disappointments illustrates the critical importance of choosing the right ligand and the right tissue for receptor binding studies. The three groups, Levitski *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2773 and 4246; 1974) Lefkowitz *et al.* (*Biochem. Biophys. res. Commun.*, **60**, 703; 1974) and Aurbach *et al.* (*Science*, **186**, 1223; 1974) all used high specific activity β -adrenoceptor antagonists as ligands, the compounds

being DL- ^3H -propranolol, L- ^3H -alprenolol and ^{125}I -hydroxybenzylpindolol respectively. All of these drugs are very potent antagonists at β -adrenoceptors, with binding constants of the order of $10^9\ \text{l mol}^{-1}$. In addition all three groups used the membranes of avian or amphibian red blood cells as the source tissues for binding studies. Such cells are known to possess a catecholamine-sensitive adenylate cyclase activity that is triggered by a β -adrenoceptor mechanism, although the physiological significance of such receptors in red blood cells remains unclear.

The binding of the labelled antagonists to the cell membranes was found to be saturable and a considerable proportion of the binding (as much as 80% in the case of Lefkowitz *et al.*) could be prevented by the addition of an excess of non-labelled propranolol or other known antagonists. Furthermore, the active D-stereoisomers of the antagonist drugs propranolol and alprenolol were more than one hundred times as potent as the inactive L-isomers in preventing binding of the labelled material. Binding could also be inhibited by other β -adrenoceptor antagonists, and by catecholamines such as isoprenaline, noradrenaline, adrenaline or dopamine, but not by α -adrenoceptor antagonists such as phen-

tolamine, or by neutral or acidic catechol substances. Isoprenaline was more potent than adrenaline or noradrenaline and dopamine was only weakly active, in keeping with the known potencies of these substances as agonists at β -adrenoceptors. The effects of the catecholamines were also stereospecific, with the biologically active L-isomers being about one hundred times more effective in preventing ligand binding than the inactive D-isomers.

Dissociation constants for the antagonist drugs in the binding assays were in the nanomolar range and were similar to those determined in these and earlier experiments for these substances as antagonists of catecholamine-stimulated adenylate cyclase activity in the erythrocyte membrane preparations. Dissociation constants for the catecholamine agonists were about two orders of magnitude higher and were again similar for the binding assay and the adenylate cyclase effects. The apparent affinities of various antagonists and agonists for the binding sites thus paralleled the biological effectiveness of these compounds on the β -adrenoceptor stimulated adenylate cyclase, a finding which lends strong support to the conclusion that the binding assays are indeed specifically detecting β -adrenoceptors. The number of such receptor sites on each turkey erythrocyte is approximately 1,000 (Levitski *et al.*) and corresponds to 0.2 pmol mg⁻¹ membrane protein in frog erythrocytes (Lefkowitz *et al.*), values that are much closer to estimates of receptor density for peptide hormones in fat or liver cell membranes than the much higher figures previously obtained in catecholamine binding studies.

Although the present reports represent the first successful identification of catecholamine receptors, it remains to be seen whether this approach can be extended to β -adrenoceptors in more complex tissues. Other catecholamine receptor sites, such as α -adrenoceptors or dopamine receptors in CNS still, of course, represent unconquered peaks.

Estonian wetlands

from Peter D. Moore

WETLAND habitats have proved particularly sensitive to the agricultural and industrial demands of man in the last two centuries. Those which have not been drained for agriculture or exploited for peat reserves have often been polluted or eutrophicated by a variety of effluents. Much international effort has been aimed recently at the conservation of wetlands, both because of their innate vulnerability and because of their immense biological

interest. Popular concern, directed chiefly towards spectacular waterfowl, has not proved difficult to stimulate.

Information on the current state of affairs in the Estonian Soviet Socialist Republic is now internationally available as a result of the recent publication in English of their 7th contribution to the International Biological Program entitled *Estonian Wetlands and their Life* (Academy of Sciences of the Estonian SSR; 1974). This collection of papers is concerned with the status of wetland habitats and wetland species in Estonia; it provides some welcome information on the conservation measures currently being invoked for their protection and on some of the fundamental scientific work in progress on the wetland reserves.

Estonia, with an area of 45,215 km², has about 1,150 lakes, covering 2,130 km² (4.8% of the country's area) and about 18,000 km² (20%) of peatlands, and some of these areas are of international importance for wildlife conservation. Conservationists in Estonia, particularly Professors E. Kumari and V. Masing, have proposed to the IUCN that twelve areas be recognised as worthy of high priority for conservation. These cover a total area of almost 90,000 ha, and four of the sites (about 28,000 ha) are already state nature reserves.

The Matsalu Bay area on the west coast of Estonia is a well-known site of ornithological interest, where greylag goose, bittern and marsh harrier breed. The mute swan, which also breeds here and is prized as a rarity (about 60 pairs in Estonia), can hardly be regarded as worthy of international interest. Matsalu Bay was raised to the status of a state nature reserve in 1957 by the direct action of the Supreme Soviet of the Estonian SSR, although wildfowling had officially been banned in the area since 1947. Despite this there are still problems, such as the influence of land management in neighbouring state and collective farms, recreational boating and sporting activities.

In a country so rich in peatlands, it is natural that these should figure prominently among the sites of conservation value. Six peatland sites, comprising over 30,000 ha are proposed for conservation, only one of which, the Nigula peat bog in south-west Estonia, is currently a state nature reserve. Many of these peat bogs have suffered less interference at the hands of man than almost any other Estonian habitat and their vegetation and structure are of particular interest. About 30% of Estonian peatlands are of an ombrotrophic type, the remainder being fens and transition mires. The ombrotrophic mires are largely raised bogs of the continental type, often be-

ing wooded with stunted pines. As one moves from the western bogs towards the east, so oceanic species such as *Myrica gale* give way to continental dwarf shrubs like *Chamaedaphne calyculata*.

The bird life of the Estonian peat bogs adds to their biological importance. Such species as wood sandpiper, whimbrel and great grey shrike breed in some numbers on these bogs, but the crane, willow grouse and peregrine falcon have declined very severely during this century, particularly during the last two decades. Between 1951 and 1961 the nests of the peregrine falcons, which were often situated on the ground in peat bogs of the Baltic region, were frequently found to contain only one egg; previously two eggs had been normal. By 1960, 80% of the breeding population had been lost and it is now considered extinct as a breeding bird in Estonia. Broken and added eggs were found during the days of the peregrine's decline, but no mention is made in this account by Kumari of the probable involvement of pesticides in this process.

The medium is the message

from Robert Shields

PROBABLY the most widely studied phenomenon in cell culture is that of contact inhibition of division. This occurs when normal cells are grown until they form a layer a single cell thick over the surface of the culture vessel, at which point (the saturation density), cell division decreases dramatically and the cells are said to be contact inhibited. Contact inhibition has come to be the *sine qua non* of normal cells, as tumour cells will often grow long after contact has been established and to far higher saturation densities—indeed the saturation density achieved *in vitro* is directly related to their tumorigenicity *in vivo* (Aaronson and Todaro, *Science*, **162**, 1024; 1968).

The role of cell-cell contact *per se* in the development of contact inhibition has been in doubt for some time, since many normal cell lines can be persuaded to reach higher saturation densities by more frequent changes of the culture medium or by increasing the concentration of serum in the cultures. These results suggested that the culture medium might be the limiting factor and that exhaustion of serum might be especially crucial. This was conclusively demonstrated by Dulbecco when he showed that the saturation densities achieved by the mouse 3T3 fibroblasts (but not the epithelial lines tested) was not related to the surface area of the culture vessel (as would be expected if cell growth was limited by cell-cell

contact) but rather to the supply of medium (*Nature*, **246**, 197; 1973). These experiments implied that cells stop growing when the medium is exhausted. But this does not explain two important observations. The first is that medium removed from contact-inhibited cultures will support the growth of non-confluent cells, showing that the medium is not exhausted. Second, if a strip of cells is removed from a confluent contact-inhibited culture, the cells at the edge of the 'wound' and those that migrate into the denuded area will divide, while the cells in the confluent cell layer which share the same culture medium will not. The 'wound' experiment was seen as the most persuasive evidence that cell-cell contact itself plays a part in contact inhibition and that contact somehow desensitises the cells to growth-promoting factors in the medium.

How does this contact-induced desensitisation occur? A possibility originally proposed by Rubin and expounded by Stoker (*Nature*, **246**, 200; 1973) is that there is a layer of medium in close contact with the cells which does not exchange freely with the bulk of the culture medium. Access of the cell layer to medium components would be limited by diffusion across this boundary layer and this limitation would lead to contact inhibition.

The diffusional flux of medium solutes across this boundary layer may be increased in two ways. One is to increase their concentration in the bulk of the medium (which would explain why the addition of increased concentrations of serum will stimulate growth in contact-inhibited cells), the second is to decrease the depth of the diffusion boundary layer. This vanishes at the edge of the cell sheet (which explains why cells in the wound can divide while those in the cell layer do not). Also the depth of the layer can be decreased by increasing the velocity of medium flow over the cell sheet: a fourfold increase in velocity will double the diffusional flux across the boundary layer—effectively doubling the concentration of medium factors reaching the cell surface (Maroudas, *Cell*, **3**, 217; 1974). This interpretation of the mechanism of contact inhibition was supported when Stoker showed that pumping medium rapidly across a confluent cell sheet will promote some cell division along the medium stream. In a more recent paper Stoker shows that the vast majority of contact-inhibited cells can be induced to divide without changing the medium (*Cell*, **3**, 207; 1974) if the velocity of medium flow across a cell layer is greatly increased by vigorous shaking of the culture.

The question that remains is: what are the crucial medium factors needed by the cell to promote cell division?

This depends on the cell type and the conditions of culture but in cases where the bulk of the medium is not depleted, diminished access to serum factors across the boundary layer is probably the cause of contact inhibition. The fact that many tumour cells do not exhibit contact inhibition may be ascribed to their relative insensitivity to depletion of serum in the culture medium, so that even at high cell densities diffusion across the boundary layer is sufficient to provide the small amounts of serum necessary for tumour cell growth. If this was the case then tumour cells selected for inability to grow in low concentrations of serum should become contact inhibited. This has been shown to be the case (Vogel and Pollack, *J. cell. comp. Physiol.*, **82**, 189; 1973).

So it seems that contact inhibition (at least in mouse fibroblasts) may be explained by the existence of a diffusion boundary layer that prevents ready access to serum factors in the culture medium rather than by cell-cell contact. Whether serum sensitivity will replace contact inhibition as the new criterion for the normality of cells remains to be seen.

Terns as predators

from our *Animal Ecology Correspondent*

THE classic model of predator-prey interaction proposed by Volterra (*Mem. Acad. naz. Lincei* (ser 6), **2**, 31; 1926) has been criticised on many grounds. Most criticisms have centred on the biologically impossible constraints concerning the reproductive rates of both predator and prey and their movements in time and space. In real life the dynamics of interactions are influenced by many factors such as a sharply defined breeding season and a time lag between the consumption of prey and the production of young as a response to it by the predator.

Maynard Smith and Slatkin have drawn attention to the likelihood that stability in predator-prey interactions is strongly influenced by yet another factor, namely the variety of hunting abilities normally found in mixed-age populations of predators (*Ecology*, **54**, 384; 1973). According to their calculations stability is maintained by the conservation of a prey species brought about by the strongly selective effects of the density of both it and its predator, and the differential in hunting ability found within the predator population. There is little doubt that the young of some species are totally ineffective hunters until they have been taught all the finer points of stalking. Remarkably little is known about the ecological effects on the prey species of such a life history strategy even with respect to the best documented species.

It is interesting, therefore, to note the work of Buckley and Buckley on the feeding ecology of the royal tern, *Sterna maxima* (*Ecology*, **55**, 1053; 1974).

Working in the Netherlands Antilles islands where royal terns spend the winter, Buckley and Buckley recorded data on several aspects of feeding behaviour. First, they observed as many dives as practicable and noted whether or not they resulted in fish being caught. If fish were dropped after being caught, this too was recorded. Second, they recorded the time budget of birds by counting the numbers of adults and juveniles on the roosts. Subtraction of this from the total number gave a measure of the time spent fishing. Third, they recorded the length of the periods when adults were fishing alone, juveniles fished alone, or both age classes together. Their results showed that adults' foraging passes lasted about half as long as did those of juveniles. Adults made 1.7 times more dives per minute as juveniles and the number of fish eaten per minute was 1.6 times that of juveniles. This was not because juveniles obtained fewer fish per dive than adults—although they frequently dropped, but recaptured, the fish—but that their diving rate was lower. These observations are consistent with the notion that fish are difficult to catch but that an increased fishing rate can offset this difficulty. The relative absence of adult terns, but not juveniles, from areas low in fish suggests further that adults are more adept at detecting prey than juveniles, and so work to a more relaxed time budget.

Some years ago Salt and Willard attempted a component analysis of predation of Forster's tern but did not, unfortunately, compare adult with juvenile fishing success (*Ecology*, **52**, 989; 1971). They suggested that searching rate by birds is inversely proportional to prey density. In the light of the Buckleys' study this should be modified to include 'within age classes'. The drop in attack rate in early spring noted by Salt and Willard was probably the result of the sudden influx of juveniles into the population and the rise of successful captures from spring through to winter an expression of a change in the structure of the prey population.

The royal tern study has demonstrated clearly that several foraging variables are subject to change with respect to age of individual and associated age structure of the predator population. Little by little reliable data are emerging which show that the accepted axioms of the Volterra approach are in need of revision. As far as terns are concerned, the cheeping of tiny beaks may be a blessing in disguise to later population stability.

articles

Differentiation of the Skaergaard Intrusion

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Re-examination of the Skaergaard intrusion of East Greenland has revealed that the model of differentiation based on an original liquid with the composition of the chilled margin and a large hidden zone is no longer tenable. The composition of the Skaergaard magma has been determined experimentally for various stages of its evolution, and these compositions are consistent with revised volumetric and compositional data for the individual units of the intrusion. In its later stages, the magma separated into two immiscible liquids, one rich in iron and phosphorus and the other rich in silica and alkalis.

THE Skaergaard intrusion of East Greenland is a prime example of an igneous body that differentiated to an extreme degree through crystal fractionation. There are probably more geological and geochemical data on the intrusion than on any other igneous body in the world; but for some time it has been recognised that there are several puzzling aspects of these data that conflict with the inferred form and pattern of differentiation of the Skaergaard magma. The fractionation scheme deduced by Wager¹ in 1960 from mass-balance calculations required a large Hidden Zone amounting to about 70% of the total volume and extending almost to the mantle. The shape and composition of this postulated Hidden Zone were unlike those of any other known igneous body and seemed to violate certain mechanical and petrological principles. In 1971, another study of the intrusion was initiated in the hope that new geophysical and petrochemical techniques might provide the added insight needed to resolve some of these questions, and expeditions to the Skaergaard region were made in 1971 and 1974. The results of some of this work have already been published^{2,3} but most is still in progress. Here I describe some salient findings of an experimental study of high temperature phase relations and their bearing on the differentiation mechanism of the Skaergaard magma.

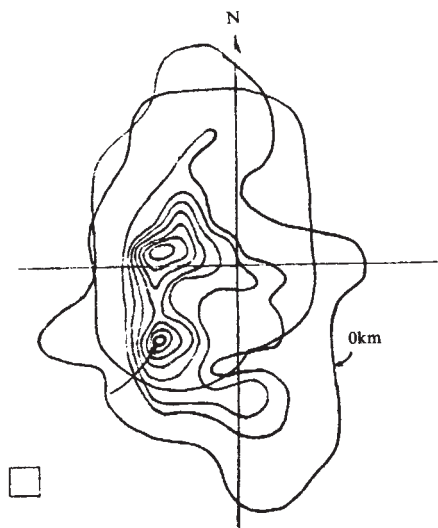
Measuring initial composition

The intrusion provides a wealth of information and is probably the only differentiated gabbroic body for which there are enough data to determine with any degree of confidence the composition and conditions of crystallisation of the evolving magma. The record of differentiation is well displayed in a 2,500-m sequence of layered gabbros. Because these rocks were formed from precipitated mineral assemblages that differed in composition from their parent liquid, there is no direct way of determining the composition of the differentiating magma. The method used by Wager¹ was based on a presumed initial composition which he took to be that of an analysed specimen of the chilled margin. Successive residual liquids were calculated by subtracting from this original composition the rocks of the Layered Series in amounts proportional to their stratigraphic thickness. The various factors used in this calculation were subject to serious errors, and the resulting calculations, though the best available, were open to question⁴.

Wager's model required a large Hidden Zone to balance the observed Layered Series and make the total intrusion have a bulk composition equivalent to the chilled margin. Gravity and magnetic surveys⁵ have produced little evidence to support that hypothesis (Fig. 1). Furthermore, new analytical data on samples of the chilled margin¹⁸ show that the composition used by Wager as the basis of his calculations was in some respects unrepresentative (Fig. 2); the same is true of the compositions of the Layered Series and Border Groups for which Wager was forced to use averages that in some cases were based on a single analysis.

Recent theoretical and experimental advances have made it possible to estimate parental liquid compositions directly from the rocks themselves. One can estimate the conditions of crystallisation from the nature of precipitated mineral assemblages and then determine experimentally the composition of liquids that are in equilibrium with these assemblages under the appropriate conditions. The Skaergaard rocks are well suited for such an approach, as there is already considerable data on the conditions of crystallisation. Temperature and oxygen fugacities for the Layered Series have been calculated⁶ from the coexisting iron-bearing phases, and the temperatures and total pressure at the last stages of crystallisation were determined from the relations of polymorphs of SiO_2 and $\text{CaFeSi}_2\text{O}_6$ (ref. 7 and Nash, unpublished). These data show that total pressure was about 500 bar at the Sandwich Horizon and about 1,200 bar at the base of Lower Zone A. During the present investigation an experimental study of the Lower

Fig. 1 Subsurface structure of the Skaergaard intrusion calculated from gravity and magnetic surveys⁵ assuming a density contrast between the gabbro and gneiss of 0.3. Contour interval is 500 m; maximum depth (arrowed) is 3.84 km; b square in bottom left is 1 km on a side.



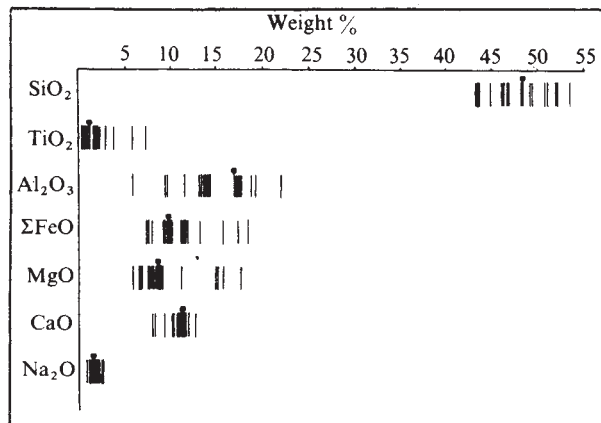


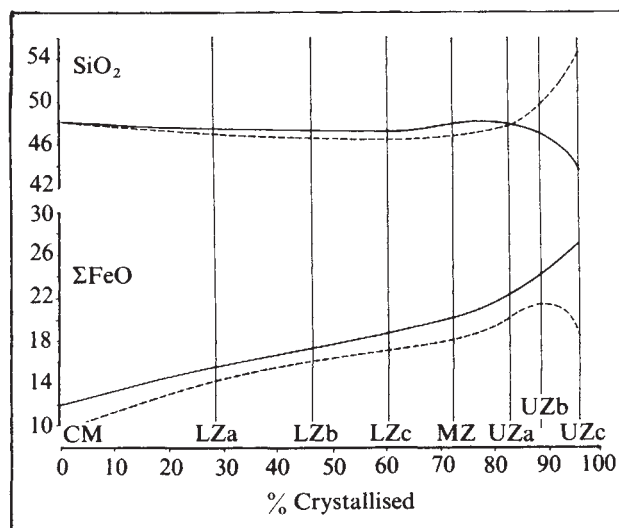
Fig. 2 Variations in the major element compositions of analysed samples of the chilled margin. Values used by Wager¹ in his calculations, shown by the small arrows, are close to the mean of the new data but do not necessarily correspond to the composition of the original liquid. Some of the observed variations result from contamination effects of the gneissic wall rocks; others probably reflect deuteritic effects, including transfer of mobile components in both directions after the chilled margin was formed. It now seems doubtful whether any sample of the chilled margin is likely to have preserved the composition of the magma making up the interior of the intrusion.

Zone showed that amphibole would crystallise at that level if water pressures were greater than about 1,500 bar. The absence of primary hydrous minerals in the intrusion indicates that this is an upper limit for the water pressure that could have prevailed during crystallisation.

Nature of the original Series

At the time the Layered Series crystallised, imperfect fractionation resulted in small amounts of liquid being trapped interstitially between cumulus crystals on the floor of the magma chamber. This trapped liquid now forms interstitial grains and overgrowths on the primary precipitates. Theoretically, it can be restored to its original liquid state if the rock is returned to the conditions of crystallisation of its cumulus phases. Rocks that are best suited for this purpose are those containing relatively high proportions of elements which are excluded from the cumulus minerals and provide a measure of the amount of trapped liquid⁸, such as Zr, Rb, Hf and K.

Fig. 3 Comparison of the experimentally determined liquid compositions (solid curves) for SiO₂ and iron oxide at successive stages of crystallisation of the Layered Series with the values calculated by Wager¹ (broken curves).



In practice several factors could intervene during crystallisation and cooling to alter the composition of any liquid that would be restored artificially, so the glass produced by quenching partially melted Layered Series rocks will differ somewhat from the original trapped liquids. The compositions obtained by this method must be tested by preparing synthetic material corresponding to the composition of the artificially restored liquid and ascertaining whether the proper minerals appear on the liquidus at a uniform temperature appropriate for the stage of differentiation at which those same minerals crystallised in the intrusion. If they do not, the liquid composition or the conditions of temperature and oxygen fugacity must be incorrect. By varying one or more of these factors the natural and synthetic phase relationships can eventually be matched.

Such a procedure of tedious trial and error adjustments ultimately yielded consistent relationships between the natural rocks and liquids that could have produced them. The solution

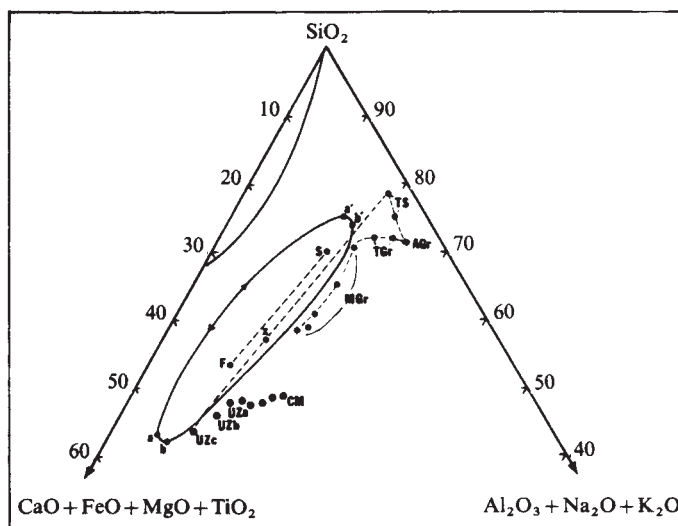


Fig. 4 Compositional relations of Skaergaard liquids. Diagram is the same as that used by Weiblen and Roedder¹⁸ except that TiO₂ has been included with the mafic components MgO + Ca + FeO. Composition x was prepared by mixing and homogenising two parts by weight of the liquid of Upper Zone C with one part granophyre of the Tinden Sill. F and S are compositions of coexisting glasses in the same sample after it was held at a temperature about 5 °C above its liquidus and quenched. a, a' and b, b' are pairs of coexisting immiscible liquids found by Roedder in the system Fayalite-Leucite-Silica. MGr, TGr and AGr are melanogranophyres, transitional granophyres and acid granophyres from the Layered Series, and TS is the granophyre of the Tinden Sill. Broken lines through the granophyre compositions indicate probable boundaries followed by liquids descending to a ternary minimum at AGr. The distinctive trend of the Tinden Sill is explained by progressive melting of gneiss with more silica than AGr.

obtained by this method is not unique, unfortunately, because there are several variables that cannot be evaluated, and if some condition has been misjudged or neglected the liquid composition obtained could be in error even though it gives consistent results. As our understanding of the conditions of crystallisation of the intrusion are improved, we can expect that better estimates for the liquid compositions will be possible.

Details of this work will be reported elsewhere, but several features of the experimentally defined liquids differ from those calculated by Wager and merit comment (Fig. 3). Instead of being steadily enriched in silica, the late stage liquids are strongly depleted in this component. On the other hand, enrichment of iron was even stronger than Wager postulated. As Chayes⁴ deduced by other means, there was little if any enrichment of alkalis.

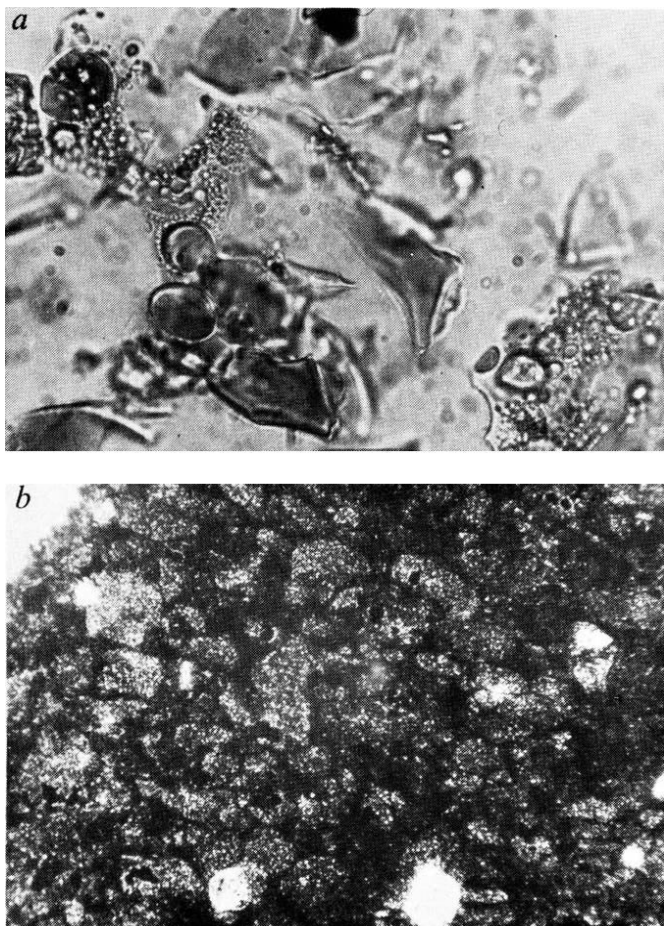


Fig. 5 Typical textures in quenched glasses produced when compositions intermediate between liquids of the Upper Zone and granophyre were first homogenised at 1,375 °C then run at temperatures just above their liquidus. *a*, Two parts granophyre and one part Upper Zone C; *b*, textures obtained under the same conditions from compositions in which the proportion of the granophyric component is small compared to that of the Upper Zone liquid. Width of fields is 0.12 mm.

Of more importance, however, is the discovery that the trend of differentiation in the Upper Zone was in part governed by an immiscible relationship. Others have speculated on immiscibility in the Skaergaard intrusion^{9,10,19}. This possibility attracted attention during the course of experimental work at the University of Edinburgh in 1972 when it was found that synthetic compositions prepared from coprecipitated gels¹¹ with compositions close to those of the Upper Zone liquids could not be homogenised at temperatures slightly above their normal liquidus. The close similarity between these liquids and those found to be immiscible in lunar rocks formed under similar conditions¹², together with the fact that the liquid of Upper Zone C and a typical granophyre had almost the same liquidus temperatures (near 1,000 °C), reinforced this suspicion. As in the case of lunar rocks, one of the conjugate liquids is rich in iron and phosphorus and low in silica while the other is rich in silica and alkalis but low in iron.

This hypothesis was thought to be untenable at one stage¹³ when difficulty was encountered in duplicating the immiscible separation in liquids that had first been homogenised at higher temperatures. It is now known, however, that this was the result of the form of the liquidus and solvus boundaries and the fact that small differences of composition may cause the contrast between coexisting liquids to be drastically reduced or even eliminated. Another factor arguing against immiscibility was the evidence of trace element and isotopic data (G. G. Goles and E. J. Dasch, unpublished) that indicates

that the large granophyric bodies within the Upper Border Group could not have been derived, at least in large part, from the same parental liquids that formed the gabbros. Instead, they have stronger affinities to the basement gneisses.

After further study that narrowed the possible errors in liquid compositions, temperatures and oxidation conditions, it has become clear that the Upper Zone liquids followed the boundary of a two-liquid field close to that outlined by Roedder¹⁴ and illustrated in Fig. 4. Liquids rich in iron that precipitated the mineral assemblages of the Upper Zone have an immiscible relationship to rocks rich in silica such as the melanogranophyres found in the same horizons and in the Upper Border Group. Compositions intermediate between these end members were prepared by repeated fusion, grinding and homogenisation at temperatures near 1,375 °C. They were then placed in unsealed silver-palladium envelopes and held for periods of 1–10 h at temperatures about 5–10 °C above their liquidus in an atmosphere controlled by mixtures of hydrogen and carbon dioxide. Oxygen fugacities were close to those estimated for corresponding levels by Williams⁶ and were calibrated against quartz-fayalite-magnetite and magnetite-wüstite reaction curves. Unmixing was observed in runs as short as 1 h, but the size of globules and compositional contrasts increased in longer runs.

Typical textures are illustrated in Fig. 5. Two distinct glasses were readily produced from compositions close to the end member rich in silica. More iron-rich compositions produced finer textures consisting of clouds of small droplets of glass and possibly cristobalite, similar to those reported by Grieg¹⁵.

Compositional relations are shown in Fig. 4. Early liquids corresponding to the Lower and Middle Zones cross the central part of the diagram as they become progressively depleted in feldspar components and enriched in iron. They reach the solvus and change their course to a trend away from silica, alkalis and alumina. The compositions of typical melanogranophyres are complementary to this trend and lie on the opposite side of the solvus. Acid granophyres seem to have evolved farther down the liquid line of descent between the solvus and an invariant point close to the $\text{SiO}_2\text{--Na}_2\text{O}+\text{K}_2\text{O}$ side of the diagram. The density contrast between the two immiscible liquids is marked (approximately 2.8 for the ferro-gabbro compared to less than 2.4 for the granophyres, according to unpublished measurements by T. Murase) and the viscosities* of the iron-rich liquids must have been low (about 10^3 poise; Murase, unpublished), so that gravity separation of two such liquids could have been efficient. Small irregularly shaped granophyric bodies are common in the upper horizons of the Layered Series and in the lower units of the Upper Border Group (Fig. 6).

Fig. 6 Typical outcrop in the upper part of Upper Zone C showing irregular felsic bodies interpreted as partially segregated immiscible liquids.



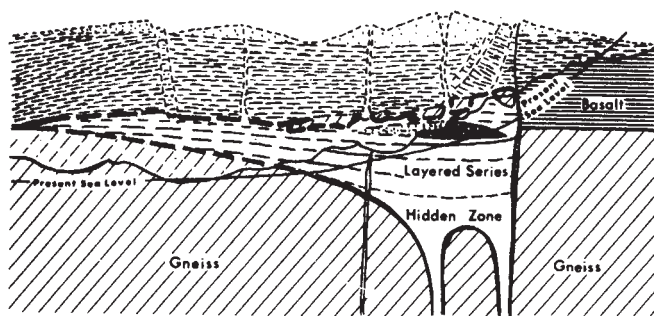


Fig. 7 A schematic north-south section through the Skaergaard intrusion showing structural and volumetric relationships consistent with known data.

As a further test of the experimentally determined liquid compositions, one can calculate the compositions and proportions of mineral assemblages that must be removed from each liquid to proceed to its successor. These fractionated assemblages should match corresponding units of the Layered Series and Border Groups for appropriate levels. When mass balance calculations of this kind are carried out for the new Skaergaard data, it is found that consistent solutions are obtained, but there is some uncertainty in the composition and relative volumes of the Border Groups, for which few data are available. For this reason, a reliable solution of the volume problem must await a thorough study of additional samples collected in 1974; using data obtained so far, it has been possible to make preliminary estimates of the relative volumes of the principal units of the intrusion. By relating these proportional volumes to measured stratigraphic thicknesses one can estimate the lateral extent of each layer and from this the form of the intrusion. Figure 7 illustrates a schematic section that is consistent with geophysical data and average compositions of the rocks and liquids as they are known at this time.

Apart from the great reduction of the volume of the Hidden Zone, the principal differences between these and previous estimates are in the greater lateral extent of the middle portions of the intrusion from Lower Zone B through the Middle Zone.

A further complication arises from the recent discovery that the phase layering of the Layered Series transgresses structural and stratigraphic horizons. The sequence of appearance of mafic phases is displaced upward in the eastern part of the intrusion relative to a section on the west side, so that cumulus clinopyroxene and magnetite appear and olivine disappears at levels that rise toward the east relative to distinctive horizons, such as the conspicuous Triple Group. The fact that the composition of plagioclase does not seem to be affected by this phenomenon suggests that the liquidus temperatures of iron-bearing minerals reflected a difference in oxygen fugacity from one side of the intrusion to the other. This feature was only recognised very recently and requires more study.

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Computer simulation of protein folding

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A new and very simple representation of protein conformations has been used together with energy minimisation and thermalisation to simulate protein folding. Under certain conditions, the method succeeds in 'renaturing' bovine pancreatic trypsin inhibitor from an open-chain conformation into a folded conformation close to that of the native molecule.

PROTEIN molecules owe their enormous functional versatility to the fact that they spontaneously fold into complicated and unique conformations determined by the particular amino-acid sequence¹. Discovering the relationship between protein sequence and conformation is a fascinating theoretical problem of fundamental importance. Most previous theoretical work has used the concept of 'local structure', in which the conformation of a short segment of polypeptide chain is supposed to depend almost entirely on the sequence of that segment. Although this approach has helped understand local secondary structure^{2,3}, it has not shown how residues distant along the chain can come together to form the overall conformation. The only promising attempt to study the tertiary folding of a

protein, in this case myoglobin, was based on the packing of cylinders supposed to represent α helices⁴. The method was not implemented on a computer and cannot be applied more generally to other proteins not built entirely from helices.

Here we tackle the problem differently. First, we simplify the representation of a protein by averaging over the fine details. This is done both to make the calculations much more efficient and also to avoid having to distinguish between many conformations that differ only in these finer details. Second, we simulate the folding of this simple structure by the combined use of convergent energy minimisation and normal mode thermalisation, which accelerate the process by avoiding the many non-productive random fluctuations that occur in nature. Tests of the procedure on bovine pancreatic trypsin inhibitor (PTI), show that under certain conditions it can rapidly reproduce the correct overall folding of this small protein molecule.

Simple representation of protein structure

Even the smallest protein (say 50 residues) is extremely complicated, with about 750 atoms and 200 degrees of freedom (single-bond torsion angles). Calculating its free energy presents severe computational difficulties, in particular when considering interactions with the rapidly moving solvent molecules and the

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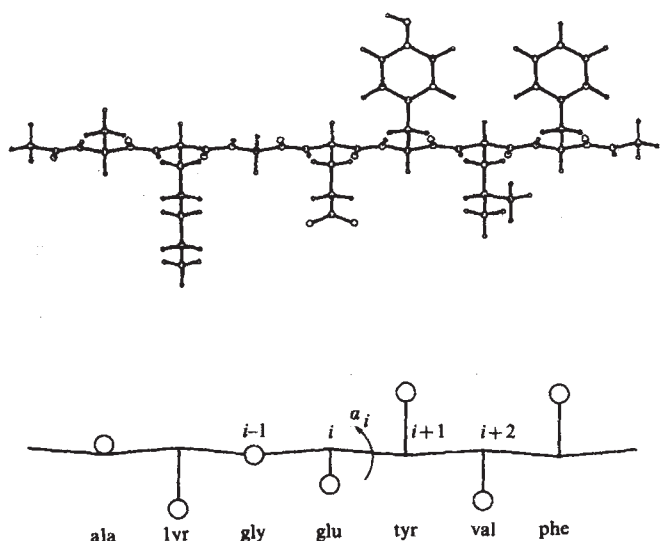


Fig. 1 Relationship between the simplified model of protein structure introduced here and the real all-atom structure of proteins. The two reference points for each residue in the simplified model correspond to the centroid of the side chain and the C^α . Each residue is only allowed one degree of freedom: the torsion angle α between the 4 successive C^α s of residues ($i-1, i, i+1, i+2$). All the side chains of a given type have the same simplified geometry. The bond lengths, bond angles, and torsion angles used to define the geometry of the simplified molecule were taken as the average values found in eight protein conformations, though they could just as well have been taken from amino-acid model compounds.

thermal motion of parts of the protein itself. Our method is designed to overcome these problems and is based on two assumptions: (1) that much of the protein's fine structure can be eliminated by averaging, and (2) that the overall chain folding can be obtained by considering only the most effective variables (those that vary most slowly yet cause the greatest changes in conformation).

Averaging over groups of atoms in the full structure gives a simplified structure with each residue represented by only two centres, the C^α atom, and the centroid of the side chain. Interactions are assumed to occur only between side chains, while the C^α positions define the chain path (Fig. 1). Each amino-acid residue only has one degree of freedom, the torsion angle about the line joining two adjacent C^α s (known here as α). Although a simple representation based on virtual bonds has been used before to study polypeptide random coils⁵, it has never been applied to ordered globular proteins. This simplification reduces the degrees of freedom by a factor of four and the number of interaction centres by a factor of fifteen. One might also hope that the reduced space used here to describe different conformations would have many fewer energy minima. The space is of lower dimension and the side chains are smooth spheres without all the minor bumps of the all-atom structures.

The effect of the fine details and more rapidly changing variables is included in the effective time-averaged potential functions used. (By the ergodic theorem⁶, this time averaging is equivalent to Boltzmann weighted spatial averaging over conformations generated by changing the fast variables.) For rotations about the torsion angle α , the effective potential is obtained by averaging the energy over all those conformations of a dipeptide that have a particular value of α . As it was impossible to study all 400 different dipeptides, calculations were done on six considered most representative: ala-ala, ala-gly, ala-pro, gly-gly, gly-ala and pro-ala. This showed that the effective potential only depended on the nature of the second amino acid, giving different potentials for the α preceding ala, gly, and pro. The alanine potential had a deep minimum at $\alpha = 210^\circ$ (twisted β chain) and a more shallow minimum at $\alpha = 45^\circ$ (α helix); the glycine potential had a

broad minimum at $\alpha = 0^\circ$ (reverse turn); and the proline potential had two sharp minima at $\alpha = 60^\circ$ and $\alpha = 210^\circ$. Because aspartic acid and asparagine were found to occur as frequently in the reverse turns of known protein conformations as glycine, the same potential was used for all three. The alanine-type potential was used for all other amino acids except proline. All the atoms were included in these dipeptide calculations, which used energy parameters derived from crystals of amino acids, amides, and hydrocarbons.

The interaction potential between a pair of identical amino-acid side chains was also calculated by spatial averaging. Each side chain was assumed to be spherically symmetrical with a radius equal to the average radius of gyration of that side chain. The effective potential was calculated at various distances apart as a sum of the interaction energy of all atoms anywhere in one sphere with all atoms anywhere in the other

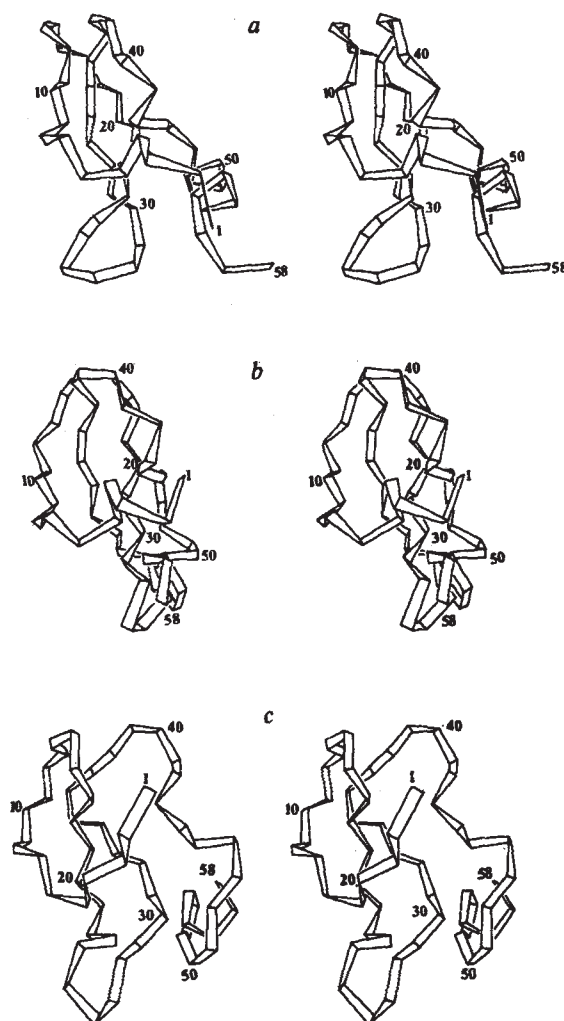


Fig. 2 Stereo ribbon drawings of PTI in: *a*, the idealised native molecule, *b*, the minimum energy conformation generated starting at the idealised native conformation and *c*, the best conformation generated by folding from an extended chain with a terminal helix (the final conformation in Fig. 4). (The programs used to rotate the molecules into the same orientation and then draw the ribbon between C^α s were provided by Dr A. D. McLachlan.)

sphere. This effective potential between identical side chains was approximated by a Lennard-Jones type function, and potentials between pairs of different side chains were obtained by a geometric mean combining law. Because proteins fold in water not a vacuum, interactions with the solvent are included by assigning to each side chain a hydrophobic energy taken from the solubilities of amino acids in water and in ethanol⁷. In the calculations, the energy of transfer between these two solvents was taken as the difference in energy of the side chain when isolated in water and when completely surrounded by other residues. When surrounded by an intermediate number of neighbours, the hydrophobic energy was varied according to a sigmoid function. More complicated models that include hydrogen bonds and S-S bridges will be described elsewhere, together with full details of the standard geometry and all energy parameters used.

The folding of this idealised protein can be simulated by solving the equations of molecular dynamics at sufficiently small time intervals. In a viscous medium like water, these equations of motion can be approximated by Langevin equations, where the change in the variables is directed down the energy gradient with a random deflection due to Brownian motion⁸. For greater computational efficiency we neglect these thermal fluctuations while the chain folds, and the end point of the trajectory is the potential energy minimum accessible from the starting conformation. We minimise the energy of the idealised protein chain with respect to all the α angles using a powerful quadratically convergent method (VA09A, by R. Fletcher and taken from the Harwell Subroutine Library). After reaching a minimum, thermal fluctuations are reintroduced and the conformation is considered to be vibrating about the minimum so that each normal mode has average kinetic energy $kT/2$ (where k is the Boltzmann constant and T the absolute temperature). A new starting conformation for the next pass of energy minimisation is chosen by suddenly stopping the thermal vibration. At this time each normal-mode coordinate will be displaced randomly from the minimum by $(R(n)kT/\lambda)^{1/2}$, so that the associated energy becomes $R(n)kT/2$. Here λ is the eigenvalue of the energy second derivative matrix corresponding to the particular normal mode, and $R(n)$ is a random number uniformly distributed between 0 and 1. (An exponential distribution of random numbers between 0 and ∞ would be more realistic.) Normal-mode thermalisation avoids non-productive changes in conformation for it knows which combinations of angle changes should cause the greatest change in conformation for a given energy increase.

Testing the simplified representation

The drastic simplifications used in the present representation of a protein conformation were tested by minimisation from near the native folded conformation. Bovine pancreatic trypsin inhibitor was chosen for this test as it is the only small protein (less than 100 residues) of known conformation that has a single polypeptide chain and no additional prosthetic group. As a first step, a simplified native PTI conformation was obtained by taking the C α positions and side-chain centroids from the X-ray coordinates⁹ (kindly supplied by Drs Huber and Steigemann). Next an idealised chain, based on the PTI sequence and having the same geometry for all side chains of the same type, was made to fit the simplified native coordinates by adjusting the α torsion angles. This conformation, known as the idealised native structure, deviates by 1.1 Å r.m.s. from the simplified native structure. The r.m.s. deviation is

$$\left\{ \frac{1}{N} \sum_{i,j} (\Delta r_{ij})^2 \right\}^{1/2}$$

where Δr_{ij} is the difference, in the two structures, of the distance between side-chain centroids (i and j). Energy minimisation from this starting conformation was then carried out to reproduce the stability of native PTI.

After 558 cycles a perfect minimum is reached at an energy of -52.0 kcalorie mol⁻¹ and a r.m.s. deviation of only 3.37 Å from the simplified native conformation. Thermal randomisation about this minimum does not lead to further movement from the native molecule on subsequent minimisation. Randomly disturbing the initial best-fit angles (with a disturbance between -15° and $+15^\circ$) has little effect on the conformation obtained by subsequent minimisation. Figure 2 compares the minimum energy and native chain folding in stereo, and Figure 3 compares the contact maps. Because of a general twisting of the molecule, the comparison of the two structures should be done in stereo. Because main-chain hydrogen bonds have been omitted, the terminal helix becomes distorted and consequently packs too tightly against the β hairpin centred at residue 27.

Simulation of PTI folding

Having shown that so simple a model can represent the stable conformation of a folded protein, we tried to simulate the actual process of folding. Most tests were done with two open

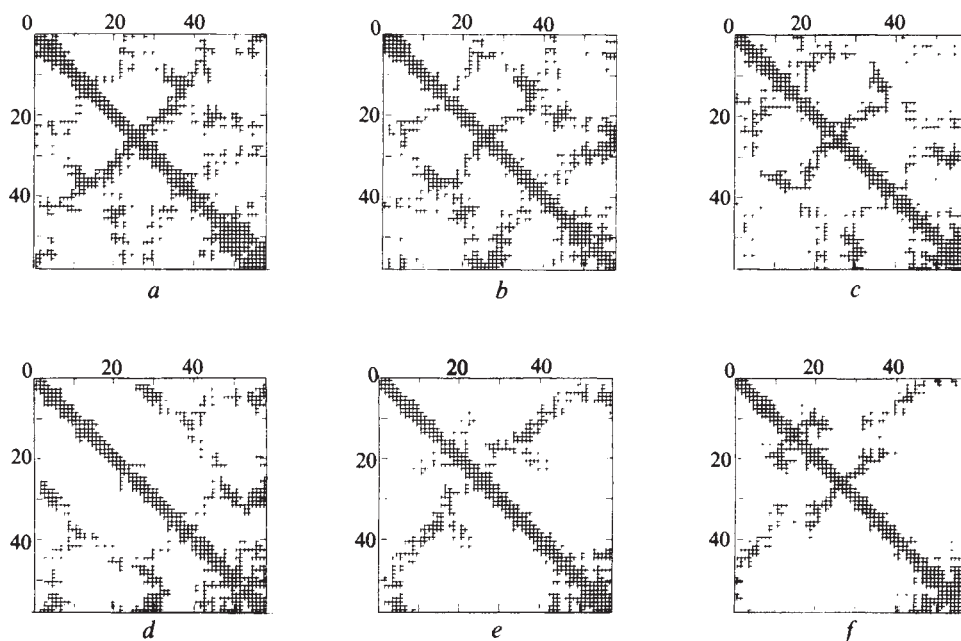


Fig. 3 Contact maps¹¹ of the following conformations: *a*, idealised native; *b*, folded from idealised native; *c*, folded from extended chain with terminal helix; *d*, *e* and *f*, folded from the same starting angles and with the same parameters as *c*, but using different sequences of random numbers for the thermalisation. The six structures shown here have energies of -332.0 , -52.0 , -48.9 , -44.9 , -32.5 , -28.7 kcalorie mol⁻¹, respectively and r.m.s. deviations from the idealised native conformation of 1.1, 3.4, 5.3, 6.3, 11.7 and 12.4 Å respectively. (The energy of the idealised native conformation is so high because it has not been minimised.) A cross at the intersection of row i and column j indicates that residue i is within 10 Å of residue j . In these maps, helices feature as a broadening of the diagonal (down from top left to bottom right), antiparallel β sheet as a band perpendicular to the diagonal, and parallel β sheet as a band running parallel to the diagonal.

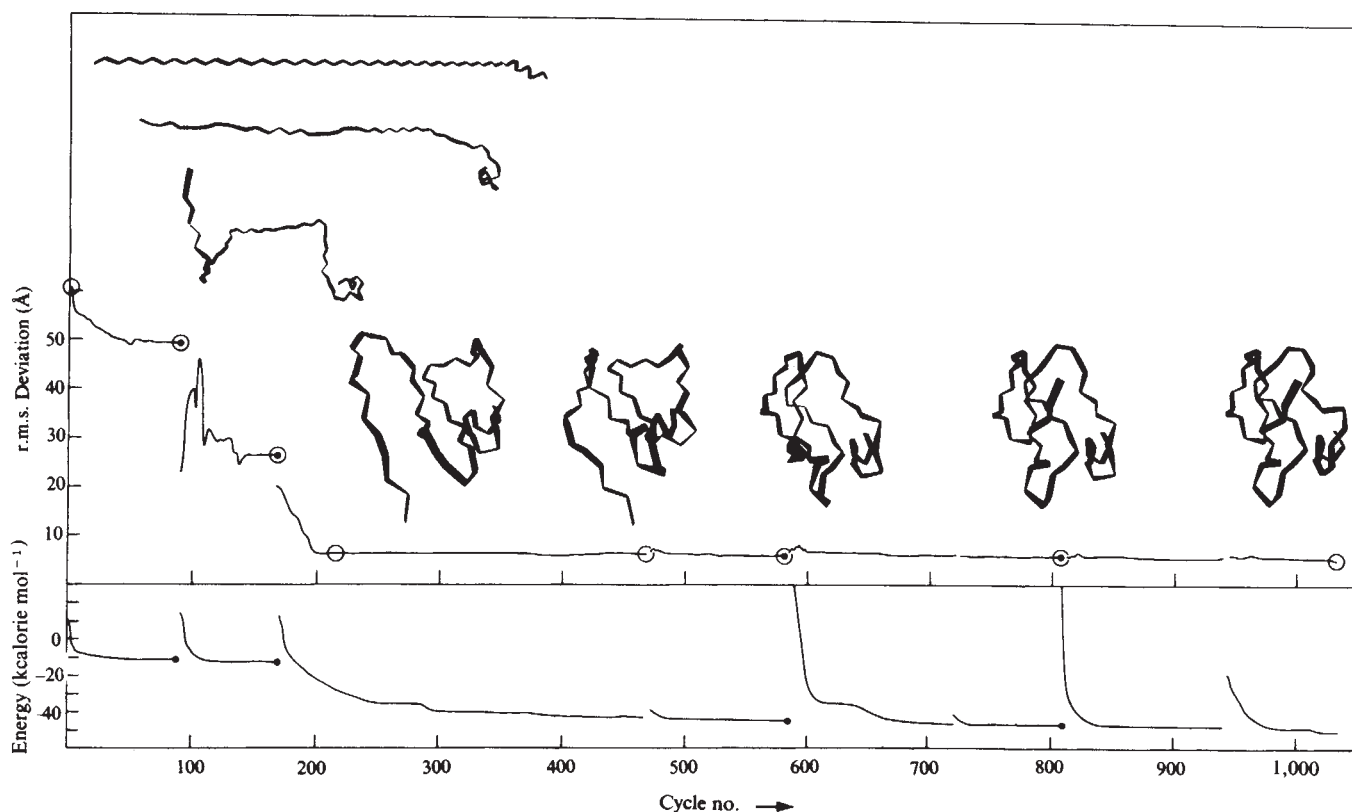


Fig. 4 Simulation of PTI folding from an extended starting conformation with the terminal helix ($\alpha = 180^\circ$ for all except 48 to 58 where $\alpha = 45^\circ$). No knowledge whatsoever about native PTI is used during this simulation (apart from setting the terminal helix). The conformation was thermalised at the end of each minimisation except near cycles 490 and 730 when the energy rises slightly because the minimisation was restarted after rounding the torsion angles to one degree. In the first two thermalisations, each normal mode was perturbed in the plus direction to raise the associated energy to $R(n)kT/2$ with $T = 1,000$ K. In the other three thermalisations, the perturbations were randomly in the plus and minus directions but always such as to raise the energy by $kT/2$ with $T = 300$ K. (Because the random numbers are distributed uniformly rather than exponentially, these temperatures do not correspond to the macroscopic temperature.) The 8 ribbon diagrams, which show the C^α chain path, refer from left to right to the 8 conformations at the circled points on the r.m.s. deviation curve, respectively. The last five conformations have progressively lower energies and are each a little closer to the native structure ($E = -43.3, -45.7, -46.0, -46.9$ and -48.9 kcalorie mol^{-1} , respectively; r.m.s. deviation = 6.08, 5.7, 5.6, 5.4 and 5.3 Å, respectively). The solid dots at the end of a minimisation indicate that a perfect minimum was reached (r.m.s. gradient less than 10^{-6} kcalorie $\text{mol}^{-1}\text{rad}^{-1}$). One cycle takes about 0.6 s on an IBM 370/165 computer.

starting conformations, one fully extended (all $\alpha = 180^\circ$), and one extended apart from the C-terminal helix ($\alpha = 180^\circ$, except for residues 48 to 58 where $\alpha = 45^\circ$). Retaining the terminal helix from the native structure is justified here as we are more concerned with the process of folding than with prediction of the native conformation of an unknown protein. In the latter case a statistical rule (see ref. 2) could be used to guess the position of the α helices in the starting conformation. Figure 4 shows the iteration history of minimisation from the second of these starting points, which was the most successful run of those obtained to date. Thermal randomisation about the first minimum, an irregular but extended conformation, raises the energy and in this case causes the chain to bend back on to itself decreasing the r.m.s. deviation. From this point, minimisation first opens the molecule again, but then reaches a new minimum where the chain now has kinks that could become the bends of β hairpins. After a second randomisation, minimisation rapidly folds the molecule bringing the terminal helix close to the β hairpin centred on residue 27. More minimisation and thermalisation first brings together the two top loops (near residues 15 and 40), and then brings the N-terminal tail on to the rest of the molecule. The final folded conformation of Fig. 4 is remarkably like the native molecule (Figs 2 and 3). In both conformations the chain bends back on itself near residues 14, 27, and 40. In both conformations, the pairs of half-cystine residues that are experimentally known to form S-S bridges, are close together (< 10 Å). It is interesting that the C-terminal helix is the part

of the native molecule reproduced least well even though these residues had been set to a perfect helix in the starting conformation; this is due to the omission of peptide-peptide hydrogen bonds which stabilise the helix and could now be introduced.

Repeating the folding simulation from the fully extended starting conformation also lead to a compact structure with many of the features of native PTI, although after the same number of cycles the r.m.s. fit was a little worse (7.7 Å instead of 6.5 Å) and the energy was higher (-27.7 kcalorie mol^{-1} instead of -44.9 kcalorie mol^{-1}). Almost all the differences in conformation of these two folded structures involved the last 10 residues which remained extended if not pre-set to a helix and consequently failed to pack against the rest of the molecule.

Variation of folding conditions

Changing either set of starting torsion angles by a random value between -15° and 15° had little effect on the final conformation. Folding at a lower initial temperature ($T = 300$ K, rather than $T = 1,000$ K) failed to reach a compact conformation, as the thermal disturbances were too small to get out of the local minima corresponding to an extended chain.

Four additional runs of 600 cycles, under the same conditions as those used in Fig. 4 but based on different sequences of random numbers, gave rise to different folded shapes. In one of these, the folded molecule was close to the native structure (r.m.s. deviation of 6.3 Å), although the β sheet was formed between parallel rather than antiparallel chains (Fig. 3f). In

two others, the antiparallel β sheet centred near residue 27 was formed, but this hairpin did not subsequently fold on to itself to give a compact shape (Fig. 3d and e). Conformations that deviated more from the native structure always had higher energies, which gives an independent criterion for choosing the best conformation and suggests that more passes of thermalisation and minimisation lead to a conformation closer to the native one. Of the five runs using different random numbers for the thermalisation step, two succeeded in getting to within 6.5 Å of the simplified native structure in less than 600 cycles. That certain folding pathways are less successful is consistent with the experimental results of Creighton¹⁰ who has analysed the predominant kinetic intermediates present at different times after starting PTI renaturation and found several with the wrong tertiary fold.

General model for protein folding?

It seems remarkable that so simple a model based on time-averaged forces can account for the stability and folding of a molecule as complicated as a protein. Looking at known protein conformations closely, one is struck by the precise geometry of the interatomic contacts that stabilise the molecule: all possible interior hydrogen bonds are well formed, and many of the nonpolar side chains interlock to form a close-packed interior. As the forces responsible for this precise geometry fall off rapidly with distance and improper orientation, it would seem that folding must depend on a very rare random fluctuation that happened to bring the right residues close together with sufficient precision for the short-range forces to take effect. It therefore seems unlikely that these short-range forces could 'direct' the folding from an open disordered structure. In view of the present results, however, the time average of these short-range forces may play an important role in directing protein folding. These effective forces, which are weak, fairly long range, and not too dependent on orientation, restrict the number of low energy conformations severely; they cause the chain to fold into the approximate shape rapidly and without having to pass through many local minima. Because this approximately folded molecule corresponds to a large region in the space of possible protein conformations, folding would not be so rare an event.

As a general model for protein folding we propose that initially, when the chain has a flexible open structure, the effective time-averaged forces between the residues play a

central role, folding the chain into a compact shape with most groups close to their final positions (say within 5 Å). Once the chain becomes compact with less freedom of movement, the specific short-range interatomic forces become important; they form a precise conformation provided that the resulting gain in enthalpy overcomes the loss in entropy. The process would be rather like crystallisation, with the atoms simply falling into place from their nearby positions in the approximate folded conformation.

To simulate this second step one switches over to progressively more detailed models gradually incorporating more atoms and ending with the all-atom structures considered in earlier work^{12,13}. Although calculating the energy of the all-atom molecule would be time consuming, one would have the great advantage of starting close to the right conformation and could minimise successive overlapping zones of a few residues at a time without having to search through many local minima.

The general concept of using a simple model based on effective time-averaged forces when the detailed forces are too complicated has many potential applications; for example, the formation of protein quaternary structure and multi-enzyme complexes, virus assembly and so on. At each level of complexity, forces would be time averaged over those substructures that are relatively fixed or seem to play a less important role in the assembly. Such a hierarchical approach might eventually lead to an understanding and simulation of very complicated biological assembly processes.

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letters to nature

Two kinds of stellar collapse

HERE I shall show that the events which produce compact objects may be divided into at least two classes on the basis of the impulse given to a binary system containing the collapsing object. This impulse may be related to the amount and symmetry of mass loss resulting from the collapse event.

If one member of a binary system in circular orbit loses mass in a spherically symmetric manner and on a time scale much less than the orbital period, then the barycentre of the remaining system acquires the velocity¹

$$S_g = \frac{M_1 M_2 (1-q)}{(M_1 + M_2)(qM_1 + M_2)} S_0 \quad (1)$$

where S_0 is the initial relative orbital velocity of the two masses,

M_1 and M_2 are the initial masses and qM_1 and M_2 are the final masses. If more than half of the total initial mass of the binary is lost the system becomes unbound; if not, the orbit becomes eccentric¹. If the initial orbit is eccentric, the explosion is asymmetric, or the effects^{2,3} of the collision of the debris with the companion star are included, then several more parameters are needed to describe the event and to determine the new orbit. Conservation of momentum arguments indicate that explosions producing loss of a few tenths of the binary mass, or giving the compact remnant a recoil velocity of several tenths of S_0 will generally (except for fortuitous combinations of parameters) lead to orbits of substantial eccentricity. If $M_1 \sim M_2$ then barycentre recoil velocities of a few tenths of S_0 will also result. Slightly more violent events produce hyperbolic orbits.

I first summarise the evidence for the expulsion of large

amounts of mass or momentum in the formation of some compact objects:

(1) The newly discovered⁴ binary pulsar is in an orbit both small ($a \sin i = 6.9 \times 10^{10}$ cm) and eccentric ($e = 0.61$). Since close binaries usually⁵ have circular orbits it is likely that the eccentricity of the orbit is the result of the recent event which produced the neutron star. An event capable of inducing this large eccentricity in a circular orbit must involve the expulsion of large amounts of mass or momentum, and will also give the barycentre a velocity of a few tenths of S_0 if the two stars are of comparable mass; since $K_1 = 198 \text{ km s}^{-1}$ I can estimate $S_g \sim 100 \text{ km s}^{-1}$.

(2) The distance from the galactic plane^{6,7} of the Her X-1-HZ Her binary system requires that if it originated in the plane it acquired a component of S_g perpendicular to the plane of at least 100 km s^{-1} . Given the observed Her X-1 mass function, a present day Population II main sequence turnoff of $0.80 M_\odot$, the assumption that to initiate neutron star formation requires $1.40 M_\odot$ of degenerate material, a Population II main sequence mass-luminosity relation and a minimum credible neutron star mass of $0.10 M_\odot$, then a simple argument⁸ shows that the age of the system is much less than the age of Population II objects, and a halo (high velocity) origin is ruled out. It is reasonable to attribute the large value of S_g to the event which formed the neutron star, although more complicated scenarios are possible.

(3) The proper motion⁹ of PSR1133+16 implies a transverse velocity $\sim 380 \text{ km s}^{-1}$. There is also evidence⁹ for high velocities in several other pulsars. This indicates either tight binding in a now disrupted binary, or an asymmetric explosion of a single star, as the origin of the high space velocities of some (or all) pulsars.

There is also evidence for a second class of compact objects, formed in a much less disruptive manner. These are the two X-ray sources 3U1746-37 and 3U1820-30, which are respectively identified¹⁰ with the globular clusters NGC6441 and NGC6624. The X-ray error boxes are 0.0184 and 0.0060 square degrees, respectively. The X-ray and optical¹¹ positions agree to about $1'$ less than the size of the globular clusters. The probability of accidental globular cluster identification of X-ray sources with such small error boxes is difficult to calculate exactly because the X-ray error boxes and cluster sizes are distributed over a wide range. A rough argument indicates that the probability is less than 10^{-4} .

The escape velocities of these globular clusters are not known. Root mean square escape velocities have been determined^{12,13} for three clusters by measuring directly the dispersion of the velocities of individual cluster stars. The results are 16 km s^{-1} for 47 Tuc, 18 km s^{-1} for M92, and 20 km s^{-1} for ω Cen, but these must be regarded as only accurate to within a factor of 2, or perhaps taken as upper limits. Mass estimates¹¹ based on an assumed luminosity function lead to r.m.s. escape velocities around 10 km s^{-1} for a representative cluster.

An X-ray source produced in a globular cluster with a barycentre velocity of 100 km s^{-1} will leave the cluster in less than 10^5 yr. The source 3U1820-30 is observed¹⁰ to vary, which supports its identification as an accretional binary source, rather than as a supernova remnant. Since the lifetime of an old population binary X-ray source is at least the Kelvin time of stars of moderate mass (Her X-1, for example, required 10^7 yr to rise to its present height above the galactic plane), the presence of a source in a globular cluster requires that it be bound to the cluster. So the events which produced the globular cluster sources were very different in character from the events producing Her X-1 and the high velocity pulsars.

No conclusion may be drawn from the presence of the massive binary X-ray sources close to the plane, because a value of S_g of only about $30\text{--}50 \text{ km s}^{-1}$ is expected from a violent explosion^{3,14}. In the expected lifetime of these systems as X-ray sources they only travel $200\text{--}350$ pc.

I denote events which produce high velocity compact objects as class A, and those producing low velocity compact objects as class B. It cannot yet be determined whether there is a continuum between these extremes. A variety of selection effects involving the observability of compact objects makes it difficult to discuss the statistics and formation rates of objects of the two classes. Most of the objects of class A are radio pulsars, and detectable only for that reason. One (Her X-1) is clearly a magnetic neutron star, but would be detected as an accretional X-ray source even were it non-magnetic, or were it a black hole. The two known objects of class B have not been reported to show regular X-ray periodicity of the Her X-1 variety. This suggests the speculation that objects of class A are neutron stars, but that those of class B are black holes into which an entire star has collapsed, without an outward-moving shock and without expulsion of matter. But calculations¹⁵ indicate that for some equations of state it is also possible to form a neutron star without expelling a supernova shell; present understanding does not justify further speculation.

The observation that two X-ray sources are in globular clusters (out of about 100 galactic sources) is remarkable even aside from the dynamical implications of their remaining bound to the cluster. The total mass of globular clusters in our Galaxy has been estimated¹¹ as $10^7 M_\odot$. This should be compared with masses¹⁶ of $2 \times 10^{10} M_\odot$ for halo Population II, and of $8 \times 10^{10} M_\odot$ for the intermediate and old disk Population II. This implies that the globular clusters have an X-ray luminosity to mass ratio about two orders of magnitude greater than that of the Galaxy as a whole, or of the central bulge which contains most of the X-ray sources.

It is clearly desirable to study the two identified X-ray sources and globular clusters further. In the 3U Catalog¹⁰ there are two more suggested globular cluster identifications: 3U1736+43 with M92 and 3U2131+11 with M15. The X-ray error boxes are large, so I have ignored these; better X-ray positions are needed.

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Origin of the optical emission from Sco X-1

Two groups have reported analyses of photometric^{1,2} and spectroscopic³ data of Sco X-1, which suggest a binary nature. Prior to these studies no persistent periodic variations of the X-ray flux have been found, but the first group¹ claims a periodic variation in the light curve of 3.93 d with a mean amplitude of 0.8 mag, and the second² claims variations of 0.22 mag in amplitude, with a period of 0.787 d.

These results are conflicting, which makes one cautious about accepting the reality of these periodic variations in the

light curve. But one can nevertheless speculate about their nature.

With the usual assumption that the visible light is emitted by a hot, optically thick plasma, which is also responsible for the X-ray emission⁴⁻⁶, it is difficult to understand these observations. The separation between the optical and X-ray emitting regions is also suggested by the simultaneous observations in these wavelengths⁷. Large changes in optical luminosity can occur while the X-ray flux remains nearly constant, although the correlations between times of X-ray and optical activity, which are not always observed, imply some connection between the two emitting regions⁷. Another striking feature is the delay of the X-ray flares with respect to the optical ones. Here I propose a model that could explain some of the observations.

I assume that we have a binary system very similar to the Her X-1-HZ Her system, that is, a degenerate star (neutron star), which is the X-ray source, and a non-degenerate star almost filling its Roche lobe. In this case, the heating of atmosphere of the non-degenerate star by the X-rays will produce a hot spot, which I assume to be responsible for most of the visible emission. I further assume, as a first approximation, that the X-ray emission is nearly isotropic.

In this case, the fraction f_x of the total X-ray luminosity absorbed by the primary star is

$$f_x = \frac{1}{2}[1 - \sqrt{1 - (R^*/D)^2}] \quad (1)$$

where R^* is the 'average' radius of the primary star and D is the separation between the components.

Similarly, the ratio y between the area of the hot spot and the total surface area is

$$y = \frac{1}{2}(1 - R^*/D) \quad (2)$$

In both formulae it is assumed that the deformation of the primary star is small.

The optical flux at maximum light, which I assume to be caused by the spot, is given by

$$\varphi_V = (L_V/4\pi)(s/y)\exp(-\tau_V)/d^2 \quad (3)$$

where L_V is the spot luminosity in the V band, s is the fraction of the spot visible at maximum light (s depends on the geometry of the orbit, mainly the orbital inclination angle) and d is the distance.

Since at maximum light the magnitude of Sco X-1 is $V = 12.2$ mag, which corresponds to a flux $\theta_V = 4.2 \times 10^{-11}$ erg cm⁻² s⁻¹ (ref. 8), one obtains from equation (3)

$$L_V = 2.4 \times 10^{34}(y/s)d_{kp}^2 \text{ erg s}^{-1} \quad (4)$$

To obtain this number I have assumed also that the interstellar absorption in the direction of Sco X-1 is $A_V = 1.5$ mag. This is consistent with counts of galaxies⁹ and also with the width of the Ca II line¹⁰.

On the other hand, if the spot radiates like a blackbody, one can write

$$L_V = 4\pi R^{*2} y \pi B_\lambda(T_e) \Delta\lambda_V \quad (5)$$

where T_e is the effective spot temperature and $\Delta\lambda_V$ is the effective width of the V filter.

Correcting the observed colours of Sco X-1 for the assumed reddening, I obtain the following range of variation

$$(U-B)_0 \simeq -1.28 \text{ to } -1.08 \\ (B-V)_0 \simeq -0.32 \text{ to } -0.28$$

These values are compatible with a spectral type varying from O5 at maximum light to B0 at minimum light.

Assuming that the effective temperature of the spot is about 37,000 K, corresponding closely to the spectral type at maximum

light, from equation (5) I obtain

$$L_V \simeq 3.9 \times 10^{34}(R^*/R_\odot)^2 y \text{ erg s}^{-1} \quad (6)$$

Now, combining equations (4) and (6)

$$(R^*/R_\odot)^2 \simeq 0.6 d_{kp}^2 \quad (7)$$

In a similar fashion we can work with the bolometric luminosity, giving

$$4\pi R^{*2} y \sigma_B T_e^4 = 4\pi f_x d^2 \varphi_x \quad (8)$$

where σ_B is the Stefan-Boltzmann constant and φ_x is the observed X-ray flux ($\varphi_x \simeq 3 \times 10^{-7}$ erg cm⁻² s⁻¹ in the 1-10 keV range). Numerically, equation (8) gives

$$(R^*/R_\odot)^2 y \simeq 5.2 d_{kp}^2 f_x \quad (9)$$

From equations (7) and (9)

$$f_x \simeq 0.117 y/s \quad (10)$$

I assume as a reasonable value $s \simeq 0.6$. Therefore, using equations (1) and (2), in order that equation (10) be satisfied,

$$R^*/D \simeq 0.45 \quad (11)$$

If the non-degenerate star nearly fills its Roche lobe, the numerical value given by equation (11) implies a mass ratio $M_2/M_1 \sim 2.2$, where M_1 is the primary star mass and M_2 is the mass of the X-ray source.

In this case, if the X-ray source is a neutron star with a typical mass of $1M_\odot$, the total mass of the system is about $3M_\odot$.

From Kepler's third law

$$D/R_\odot \simeq 4.2[(M_1 + M_2)/M_\odot]^{1/3} P^{2/3} \quad (12)$$

where P is the orbital period in days.

Accepting that $P \simeq 0.78$ d (ref. 2), equations (12) and (11) yield, respectively $D \sim 5.1R_\odot$ and $R^* \sim 2.3R_\odot$.

In this case, from equation (7) one obtains a distance of about 2.3 kpc for Sco X-1 and a luminosity in the X-ray region of about 1.7×10^{38} erg s⁻¹. This value indicates that the luminosity has reached the Eddington limit and that radiative forces play an important role in the accretion flow.

On the other hand, if we accept the results reported by Martynov¹ ($P \simeq 3.93$ d) equations (12) and (11) give values for the separation between the stars and for the primary radius which are a factor of three greater than those obtained with the data of Gottlieb *et al.* These values imply a distance greater by a factor of three and an X-ray luminosity greater by an order of magnitude. In this case, there is a difficulty since the calculated luminosity is greater than the Eddington limit.

Therefore, this model implies that orbital periods of less than 1 d are required. Gribbin¹¹ has suggested that Sco X-1 could be similar to the WZ Sge system, predicting an orbital period of about 4 h.

The bolometric spot luminosity is about 5.3% of the X-ray luminosity, $\sim 9.4 \times 10^{36}$ erg s⁻¹. Since the effective temperature is high, the ultraviolet radiation from the spot is strong enough to ionise the interstellar medium to the extent that the emission measure is 33 cm⁻⁶ pc (ref. 12) if the interstellar gas density is about 0.8 cm⁻³ in the vicinity of Sco X-1. This emission measure compares well with the value obtained by Johnson¹³ from H β measurements around Sco X-1 and solves the problem of its theoretical explanation; Silk *et al.*¹⁴ were not able to explain it by assuming that the ionisation is caused by X rays.

On the other hand, the model faces other difficulties such as the observed delay between optical and X-ray flares. These observations eliminate the possibility that the optical flares

arise from the relaxation of the atmosphere after irradiation by X-ray flares, as the model would predict. One might also suppose that the optical flares would be produced near the Lagrangian point, by instabilities in the mass flow induced by the X rays. These instabilities will later reach the X-ray emitting region, producing X-ray flares. But the observed time delay requires a propagation velocity of the instabilities of about 10^4 km s^{-1} , which is too high.

Although these and some other observed features cannot be explained, the model has some merits in predicting the masses and dimensions of the system as well as its distance. The calculated parameters are very similar to those of the Her X-1-HZ Her system, possibly indicating the existence of a class of binary X-ray sources associated with Population II objects.

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An ancient lunar magnetic dipole field

SINCE the landing of the first Luna spacecraft it has been known that the present magnetic moment of the Moon is negligible. Later, the Explorer 35 data¹ gave an upper limit of 4γ ($\sim 4 \text{ nT}$) to the dipole field at the surface. Now, Russell *et al.*² by analysing the Apollo 15 subsatellite magnetometer data obtained during passages of the Moon through the geomagnetic tail (where the magnetic field is reasonably steady) have calculated an upper limit of 0.05γ . They have shown that both the induced and the permanent magnetic dipole moments can be determined by separately analysing data obtained during passages of the Moon through the north and south lobes of the tail, in which the field is directed towards and away from the Sun, respectively. Only the permanent dipole component in the

plane of the subsatellite orbit is determinable and is less than $1.3 \times 10^{18} \text{ gauss cm}^3$: it is unlikely that the component perpendicular to it is appreciably greater. Russell *et al.*² estimated the error to be $1.33 \times 10^{18} \text{ gauss cm}^3$, and therefore concluded that the lunar magnetic moment is not significantly different from zero. Paradoxical as it may seem, it follows from this observation that the Moon possessed a magnetic field of internal origin in its early history.

Stable remanent magnetisation discovered in lava samples collected during the Apollo 11 mission has been interpreted³ as thermoremanent magnetisation acquired when the lava flows cooled through the Curie point 3.6 Gyr ago. It was concluded³ that a general lunar magnetic field of $\approx 1,000 \gamma$ was responsible. As I had proposed in a theory of the non-hydrostatic lunar figure⁴ that the Moon possessed a small iron core of 150–500 km radius, it seemed reasonable to suggest that a dynamo process within this core accounted for the field (Fig. 1a). The disappearance of this magnetising field can be explained⁵: either the magnetic Reynolds number became subcritical or the core solidified during the last 3.2 Gyr.

To assume an iron core implies that the accreted Moon was heated to its melting point soon after its origin. It is not clear, however, that there was a sufficient heat source but it is possible⁶ that the Moon had formed cold and was not heated at depth in its early history so that the disseminated iron did not sink to form a core. Thus, the Moon, forming within a gas sphere, could have acquired a uniform permanent magnetisation (Fig. 1b) from a primaevial field in the condensing solar nebula—a remnant of the galactic magnetic field lines which had not diffused away as the contracting dust cloud compressed the field. Such a general magnetic field within the Solar System would have vanished completely from interplanetary space when the gas was expelled from around the terrestrial planets during the T-tauri phase of the Sun. The postulated permanent magnetisation of the deep interior of the Moon would have produced a dipole field at the lunar surface which magnetised both the anorthositic highlands as they cooled after differentiation and the basalts after they solidified in the maria basins. The present absence of a lunar dipole field can be explained⁶ by showing that the disseminated radioactivity within the Moon heated the deep interior above the Curie point of iron (780°C) between 3.2 Gyr ago and the present.

Russell *et al.*² have concluded from their finding of so small a magnetic dipole that this theory⁶ is highly improbable, for they suppose that it is unlikely that all traces of the ancient field could have vanished so completely: they prefer to invoke dynamo action in the lunar core, which could now have ceased generating. They have calculated² the thicknesses of hypothetical spherical shells of uniform magnetisation necessary to explain the observed moment: 300 m for a remanent magnetisation intensity of $10^{-4} \text{ gauss cm}^3 \text{ g}^{-1}$ (that of many returned samples), 11 m if an intensity equal to that for the deep interior on the theory⁶ outlined here is assumed, taking

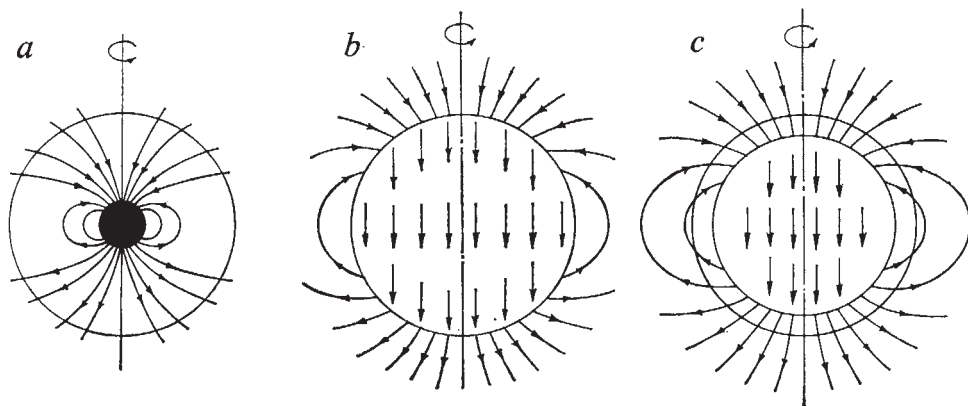


Fig. 1 The ancient lunar magnetic field. a, On the core dynamo theory; b, on the primaevial magnetisation theory: 4.6 Gyr ago; c, on the primaevial magnetisation theory: at the time of magnetisation of the Apollo rocks, after the outer shell has been heated above the Curie point.

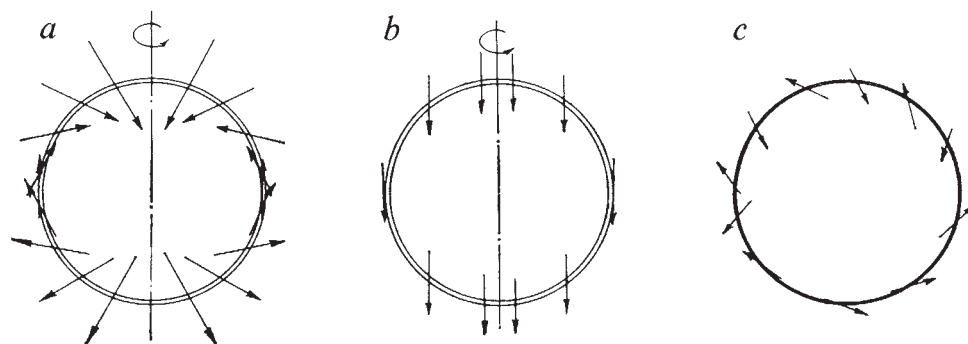


Fig. 2 Models of the magnetisation of the lunar crust; *a*, uniformly magnetised crust produced by an external magnetic field; *b*, as magnetised by an axial dipole field either of core dynamo or primaeval magnetisation origin; *c*, magnetisation in a very thin shell produced locally by impacts.

the value of the palaeofield (1.2 gauss at 3.9 Gyr ago) determined by Stephenson *et al.*⁷. They concluded that these thicknesses are implausibly small.

But the temperature rose to about 1,000° C in the outer parts of the Moon, say to a depth of 200 km, very soon after its origin 4.6 Gyr ago, the highlands forming by differentiation. Subsequent heating of an outer shell of the Moon to about 1,200° C between 3.9 Gyr and 3.2 Gyr must be postulated to account for the production of the magma which then flooded out into the great impact basins formed about 4.1 Gyr ago. The heat sources for these events are still the subject of debate, but electromagnetic and gravitational sources were available: these would not have heated the deep interior of the Moon in the first 1.5 Gyr, when the remanent magnetisation of the Apollo rocks was acquired, but the initial primordial magnetisation of the outer 300 km of the Moon would by then have been removed completely (Fig. 1c).

Thus, on neither theory of an internal lunar magnetic field could even a minute part of the original, general lunar magnetic field be expected to exist today: such fields as now exist must originate in the remanent magnetisation of those rocks which lie above the Curie point isotherm, say, 100 km deep.

In their calculations Russell *et al.*² assumed the model shown in Fig. 2*b* whereas the magnetisation of the outer parts of the Moon, acquired on the formation of the highlands and mare lavas—and possibly of deeper parts of the crust as they cooled after the major thermal events—will be directed along the dipole field directions shown in Fig. 2*a*. It is known that the intensity of thermoremanent magnetisation is proportional to field strength for small fields. Thus, the intensity of magnetisation of the spherical shell is $-c\nabla V$ where $c \approx 10^{-4}$ (assumed constant) and V is the magnetostatic potential of the magnetising field. The vectorial dipole moment $\mathbf{M}$ of the magnetised spherical shell is given by:

$$\begin{aligned}\mathbf{M} &= -c \int_V \nabla V dv, \text{ where } dv \text{ is a volume element} \\ &= -c \int_a \nabla V nda, \text{ where } da \text{ is a surface element and } \mathbf{n} \text{ is the unit}\end{aligned}$$

outward vector normal to the element at (r, θ, ϕ) .
If the magnetising field is a centred axial dipole of moment p ,

$$\begin{aligned}\mathbf{M} &= -c \iiint \left[\frac{p \cos \theta}{r^2} \right] \mathbf{n} r^2 \sin \theta d\theta d\phi \\ &= -\frac{c}{2} \left[\iint_1 p \sin 2\theta \mathbf{n}_1 d\theta d\phi + \iint_2 p \sin 2\theta \mathbf{n}_2 d\theta d\phi \right]\end{aligned}$$

where suffixes 1, 2 mean the integral is taken over the outer and

inner surfaces of the shell, respectively. As $\mathbf{n}_1$ and $\mathbf{n}_2$ at corresponding points (θ, ϕ) are opposite in sign it follows that

$$\mathbf{M} = 0$$

If the magnetising field potential is of the form $S_n(\theta, \phi)/r^{n+1}$ where $S_n(\theta, \phi)$ is a surface harmonic of degree, n , resulting from an internal source, or $r^n S_n(\theta, \phi)$ resulting from a source external to the Moon, then $\mathbf{M} = 0$ as the integrals over the two surfaces vanish separately, except that in the latter case, if $n = 1$, $\mathbf{M} = cv\mathbf{H}$ where v is the volume of the shell and $\mathbf{H}$ is the uniform field produced by an outside source, such as the Earth or Sun.

Thus, for a shell of any thickness magnetised by a dipole at its centre, the latter field being subsequently removed (Fig. 2*a*), the shell gives rise to zero dipole moment, because the polar regions, as shown are exactly compensated by the greater volume of more weakly oppositely magnetised material in the equatorial regions (Fig. 2*a*). I have also shown that all harmonics of the field external to the shell completely vanish⁸.

Thus, it does not follow from the new² limit to the dipole moment that a negligible thickness of the lunar crust is magnetised. Had this been so it may have been possible to explain the magnetisation by appealing to a yet unclarified process involving stresses in the surface rocks produced by meteoritic impact and the transient magnetic field of the solar wind locally amplified by vaporisation^{9,10}. This magnetisation would be random and confined to an exceedingly thin shell (Fig. 2*c*). But if that is accepted it becomes exceedingly difficult to explain the magnetic anomalies of 1γ, determined by the Apollo 15 subsatellite¹¹; the 'magcons' inferred from disturbances recorded by the Explorer 35 satellite¹². Such anomalies will result from the large craters in the magnetised crustal layers¹³, but as they arise from the leakage of lines of magnetic field from the edges of major topographical features, the effects would not be seen at heights of 100 km if the crust were only magnetised to a depth of 300 m. Russell *et al.*¹⁴ postulated instead that there are thick 'islands' of magnetised material in the basins, but their origin is obscure. I conclude that the small magnetic fields observed outside the Moon arise from the departure of the magnetised shell from the idealised model, caused particularly by the large craters.

Sceptics may wonder if the subsatellite magnetometer experiment has not simply proved the truth of a simple—but apparently previously unknown—theorem about potentials. I think, however, that the importance of the null result of the search for a present lunar magnetic dipole field is that it gives strong support to the theory that the natural remanent magnetisation of the lunar rocks requires the existence in the early history of the Moon of a magnetic dipole field of internal origin. The alternative hypothesis that the field was of external origin has been widely canvassed¹⁰. The possibility that this was the terrestrial field has been considered^{3,5} but was rejected on the grounds that the (tidal interaction) dynamics of the Earth–Moon

system precludes the Moon being within the magnetosphere for a long enough time to explain the magnetisation of the Apollo rocks. There are also arguments^{3,5} against an external field of solar origin being the magnetising agent. But the early history of the Earth-Moon system is uncertain and the magnetic fields of the early Sun and solar wind are unknown, so that new evidence² which excludes an external magnetic field as the magnetising agent of the lunar rocks is a major advance.

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Effect of thermal metamorphic conditions on mineralogy and trace element retention in the Allende meteorite

HEATING carbonaceous chondrite from the Allende meteorite in a low pressure environment causes visible mineralogical alteration at 700-1,000°C but not at $T \leq 600^\circ\text{C}$. Samples heated for 29 d at 500°C lose trace elements (Bi, In and Tl) more effectively than those similarly heated for 7 d. At 1,000°C with $\sim 10^{-5}$ atm initial pressure of O_2 , H_2 or He these elements, Ga and Se are comparatively more completely lost.

Chondritic meteorites provide valuable information about the condensation and early evolutionary histories of solid objects in the inner Solar System. Primitive chondrites probably reflect to some degree the condensation process(es) of solid material from the gaseous nebula and are particularly important in these respects. We take such chondrites¹ as comprising grades 1-4 of the accepted chemical-petrologic classification². These chondrites differ from their congeners of grades 5 and 6 in many respects

including their higher contents of volatiles, such as the trace elements Bi, In and Tl (refs 3-10) postulated as cosmo-thermometers⁹⁻¹². These differences could be primary (chiefly arising from differences in condensation history⁹⁻¹⁴) or secondary (chiefly arising from thermal alteration of appropriate primitive chondritic material¹⁵⁻¹⁹). Both processes probably occurred and it is desirable to evaluate the relative importance of each in evolving primitive material.

Until recently there had been no empirical attempts to determine effects on trace elements of simulated metamorphism (artificial annealing) of any primitive chondritic material. In carrying out such a study by heating samples of Allende C3 chondrite at 100°C increments over a reasonable metamorphic temperature range (400-1,000°C) in a low pressure environment (initially $\sim 10^{-5}$ atm H_2) we found substantial changes¹. After 1 week we observed minor loss of Ga and Se and major loss of the postulated cosmo-thermometric trace elements, but no loss of Co. This heating time is short compared with geological time; nevertheless two-element correlation patterns involving Bi, In and Tl retained in heated Allende samples are similar to those for E3-6 chondrites¹, indicating possible metamorphic effects in enstatite chondrites.

As there is no consensus^{9-11,15-19} on ambient meteoritic metamorphic environments, quite probably our experiments do not duplicate effective conditions in meteoritic parent bodies or proto-planets. As a tentative step in the direction of establishing differences in trace element retention arising from variations in ambient conditions we have conducted similar experiments on Allende samples using O_2 or He rather than H_2 and also extended the heating period using H_2 . These gases were chosen because of their very different chemical properties and their high cosmic abundances. Here we report these results together with a mineralogical and petrographic description of the heated samples.

Each heating run included chips (from a large individual, USNM-3643) and aliquots of a single batch of <100-mesh homogenised powder. We placed samples (350 mg) of powder and 2-4 chips (for trace element analysis), a 300 mg chip (for mineralogy and petrology) and 100 mg of powder (a reserve sample) into separate quartz vials which we sealed on to the pre-cleaned heating apparatus¹. After evacuation, the system was purged with the appropriate gas (H_2 , He or O_2), re-evacuated to $\sim 10^{-5}$ atm and sealed. The end with the samples was then placed into a muffle furnace preheated to 500°C or 1,000°C (Table 1). Condensable material liberated during heating was collected in a trap cooled with liquid nitrogen; the ambient gas pressure (as monitored with an ion gauge) jumped to ≥ 1 torr immediately on insertion of the apparatus into the muffle furnace but within 6-17 h the pressure reached a value which remained constant throughout the remainder of the run. After seven or 29 d we removed the sample assembly, quenched the vials in H_2O and sealed them off.

Table 1 Trace element contents of unheated and heated Allende samples

Heating temperature (°C)	Heating time (d)	Ambient atmosphere Initial gas	Initial pressure (mtorr)	Final pressure (mtorr)	Sample form*	Co (p.p.m.)	Ga (p.p.m.)	Trace element contents Se (p.p.m.)	Bi (10 ⁻³ p.p.m.)	Tl (10 ⁻³ p.p.m.)	In (10 ⁻³ p.p.m.)
unheated	—	—	—	—	powder	612 ± 10†	6.27 ± 0.30†	9.34 ± 0.16†	49.0 ± 0.7†	59.6 ± 2.2†	35.6 ± 1.0†
500	7	H ₂	30	175	powder	642‡	—	9.2‡	30.2‡	23‡	37.3‡
					chips	650‡	—	9.2‡	35.7‡	34‡	37.9‡
500	29	H ₂	1	36	powder	630	6.3	9.5	20.6	11	30.4
					chips	629	6.2	9.6	25.5	14	31.6
1,000	7	H ₂	20	320	powder	624‡	5.7‡	7.9‡	6.13‡	4.3‡	3.67‡
					chips	602‡	5.9‡	8.1‡	7.66‡	6.1‡	4.15‡
1,000	7	O ₂	5	240	powder	615	6.2	9.2	49.5	13.8	6.49
					chips	620	6.3	9.4	—	28.4	14.1
1,000	7	He	5	200	powder	630	4.8	5.1	10.3	2.3	≤ 4.7
					chips	651	6.1	9.6	26.7	2.4	6.10

* Powder samples were aliquots of a single homogenised batch; chips were taken from a large individual.

† Mean values of five determinations. Uncertainties listed are one estimated standard deviation from the mean calculated from the dispersion of the individual measurements¹.

‡ Data reported elsewhere¹.

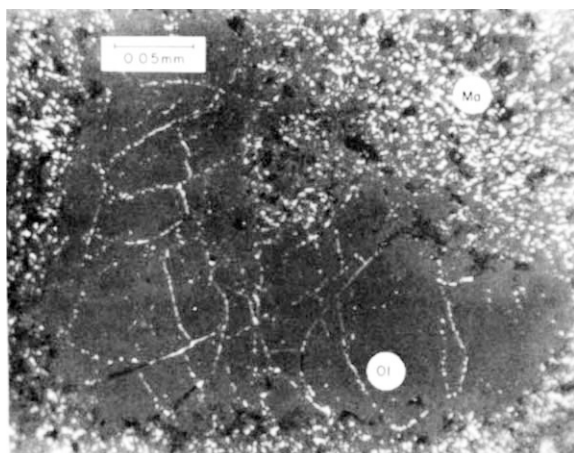


Fig. 1 Reflected light photomicrograph of Allende meteorite heated at 1,000°C, initially in H_2 atmosphere. Metallic iron particles rich in nickel are evident in matrix (Ma) and in fractures in the large olivine (Ol) crystal. Samples heated at 1,000°C, initially in He or O_2 atmosphere, seem essentially identical, implying insufficient O_2 to oxidise much of the metal formed by sulphide decomposition.

Heated samples for mineralogy and petrology and an unheated sample were prepared as polished thin sections and examined under transmitted light and reflected light. In addition, compositions of the mineral phases in several of the sections were determined by electron microprobe. We irradiated samples for trace element analysis in the Argonne CP-5 reactor for 1 week and processed them to separate Bi, Co, Ga, In, Se and Tl which were counted as described elsewhere¹. R. N. Clayton determined the $^{18}O/^{16}O$ ratios in many of our reserve powder samples and concluded that these have not been altered measurably by our heat treatment.

The samples studied, their oxygen content (in weight per cent) and the $\delta^{18}O$ (SMOW) (per mille) are: unheated (35.1) 1.1; another Allende whole rock sample (36.0) 1.5; 500°C (H_2) 29 d (34.6) 1.4; 600°C (H_2) 7 d (34.5) 1.4; 900°C (H_2) 7 d (34.0) 1.2; 1,000°C (H_2) 7 d (34.6) 1.2; 1,000°C (O_2) 7 d (34.1) 1.5; 1,000°C (He) 7 d (34.6) 1.4.

Using transmitted light we observed no visible recrystallisation effects for the transparent minerals in any of the heated samples. In all cases we found sharp crystal boundaries and well preserved textures identical to those in unheated samples. We also tried to observe effects resulting from Fe^{2+} diffusion within and between olivine and pyroxene grains. Because of the small sizes of the heated samples (~ 3 –4 mm) and the variability of the Allende samples in general, we could obtain no meaningful data for parameters such as mean iron content or percentage mean deviations (see refs 18, 19). Qualitatively, however, there was no indication of olivine or pyroxene compositions becoming more homogeneous—primary zoning was present and seemingly unaltered even for the 1,000°C run. In addition, we looked for and did not find evidence of Fe^{2+} diffusion into Fe-deficient olivine ($Fa < 1$ –2) as a result of our heating experiments. In particular, where Fe-deficient interiors of broken, zoned olivine crystals were adjacent to Fe-rich matrix there was no evidence of a diffusion profile of Fe^{2+} into the olivine.

In reflected light the unheated sample and those heated to 400°–600°C were essentially identical; but samples heated at 700°–1,000°C were markedly different. In those samples we observed no sulphide, originally present mainly as pentlandite— $(Fe,Ni)_9S_8$ —and as troilite— FeS (ref. 20). Instead we observed disseminated metal. This metal is particularly well developed in the samples heated at 900°C and 1,000°C (irrespective of

original ambient atmosphere all three 1,000°C samples seem identical after heating), occurring both disseminated throughout the matrix and along fractures in larger silicate crystals (Fig. 1). Microprobe analyses of the olivine ($Fa \sim 8$) adjacent to metal-containing fractures in the grain shown in Fig. 1 showed no decrease in Fe^{2+} content toward the fracture, indicating that the metal did not form locally by reduction of Fe^{2+} from the adjacent silicate. Instead, the metal apparently migrated inwards either by surface diffusion or vapour transport. The metal itself, in both matrix or fractures, contains 25–30% Ni, appropriate for derivation by decomposition of the sulphides. (If the metal in the fractures had formed by reduction of Fe^{2+} in the silicate, it should be Ni-deficient since the Ni^{2+}/Fe^{2+} ratio in meteoritic olivine and pyroxene is typically very low.)

Not surprisingly, the absence of discernable sulphides in samples heated at 700°C and above corresponds to the presence of off-white solid material (in the cold traps of these runs) which in one case at least was identified mass-spectrometrically (by M. Gay) as S_8 . Thus, between 600°C and 700°C, for the conditions prevailing in our experiments, sulphides in Allende samples seem to decompose ultimately to metal and sulphur. These are not the products expected by heating pentlandite or troilite in equilibrium with vapour at these temperatures²¹, but our system is open to loss of sulphur or volatile compounds of sulphur. This raises some question as to the applicability of our results to possible metamorphic models for primitive bodies since even the most recrystallised chondrites (such as Type 6) contain abundant troilite or other sulphides. But experiments in progress on enstatite chondrites show that some sulphides can be stable to higher temperatures ($\sim 900^\circ C$) and that trace element losses still occur at lower temperatures. This provides additional support for the idea that decomposition of sulphides is not prerequisite to the effects observed in samples from the Allende meteorite.

The absence of observable Fe^{2+} diffusion in any samples indicates that the diffusion rate for ferrous iron in olivine and pyroxene at the temperatures of our experiments is too slow to be observable over periods as short as a week. But this does not preclude Fe^{2+} diffusion being the principal mechanism by which uniform olivine and pyroxene compositions are produced in equilibrated ordinary chondrites^{18,19} since presumably much longer times were available.

Since the fractional loss of trace elements in Allende samples seems to depend on kinetics rather than thermodynamics¹ we were not surprised that Bi, In and Tl are lost to a greater extent from Allende samples heated for 29 d than from corresponding samples heated for 7 d at 500°C (Table 1). Relative to unheated material, In is not lost in 1 week but is lost to a minor extent by more extended heating at 500°C; Co, Ga and Se seem unaffected even in 29 d (Table 1).

The nature of the gas initially present in the heating apparatus markedly influences trace element retentivity at 1,000°C (Table 1). Relative to corresponding samples heated initially in H_2 , samples heated initially in O_2 retain the five mobile elements to a greater extent while those heated initially in He lose most of them more readily—Bi is exceptional in that it is somewhat better retained in He than in H_2 . Selenium and, more surprisingly, Bi are quantitatively retained in the O_2 case; in the He case we could establish only a conservative upper limit for In. In each case the ambient atmosphere was present at nanomolar levels, negligible compared with the amount of sample present. So it seems unlikely that differences in retentivity of the trace elements can be due to their forming or failing to form volatile chemical compounds with the gas. (Indeed, as noted, irrespective of the original atmosphere the mineralogy of the three 1,000°C samples seem identical. In particular, disseminated metal formed in all three cases indicating that the internal atmosphere could not have been extensively oxidising.) It seems more likely that both O_2 and H_2 were adsorbed on to the samples, retarding loss of the mobile elements to a greater or lesser extent. Helium would not be troublesome in this regard and indeed loss of trace elements is generally greater using this gas.

To the extent that Allende meteorite can be used as a model for other primitive materials our results have several implications relative to retention of mobile trace elements during metamorphism in primitive parent bodies. As suspected^{1,15-19} such elements can be lost by diffusion even before their host mineral(s) undergo(es) chemical or phase change. Since the duration of our experiments is short compared with geological time, our results can provide only a lower limit to the loss of mobile elements in an open system in the 'real world.' But the results clearly indicate that kinetics rather than equilibrium could play a major role in determining trace element distribution in equilibrated or partially equilibrated chondrites¹. Further, primitive meteorites differ in the amount and kind of gases or readily volatile major components they contain. Trace element retentivity also is clearly influenced by ambient atmosphere and specific regions in primitive parent bodies could conceivably be gas tight. Future experiments should allow for this possibility as well as systems completely open to gas loss. Furthermore, it seems necessary to conduct additional experiments (which are planned) for studying retentivity as a function of gas fugacity. Finally, in the event that material from other solar system objects is thermally sterilised before return to earth²² it will be necessary to choose ambient temperature and atmospheric conditions carefully so as not to degrade the sample unnecessarily. By that time we will have considerably more information on the response of other sorts of primitive material to thermal alteration.

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The Laschamp geomagnetic 'event'

SHORT lived geomagnetic 'events' provide useful stratigraphic markers; they may enhance climatic and evolutionary changes^{1,2} and they provide stringent parameters for geomagnetic models. During such events the virtual geomagnetic pole undergoes excursions outside the usual range of secular variation but they are usually brief, generally lasting about 10^5 yr, so that their

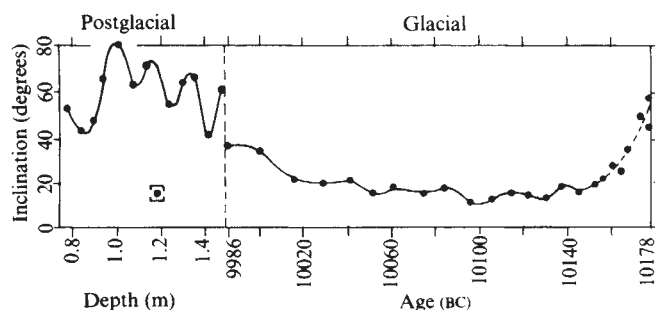


Fig. 1 Inclination of remanence after demagnetisation in an alternating field for samples from the Stårnø core. The rapid change in inclination before 10160 BC reflects the influence of water currents on the depositional remanence; the currents diminish rapidly as a consequence of glacial recession.

magnetic record in deep sea sediments is often lost because of post-depositional remagnetisation³ and because of biological activity in the upper few decimetres of the sediments⁴. In lake sediments, where sedimentation is more rapid, the magnetic record is better preserved. Dating is, however, difficult because secular variations in atmospheric carbon isotopes and irregular distributions in the environment are known to produce errors. Furthermore, geomagnetic events, the levels of which may have been displaced by bioturbation⁴, are usually dated by assuming uniform sedimentation between ¹⁴C dated horizons. The Laschamp event, in particular, seems to have been detected at 12 locations although the reported age (Table 1) varies between 7,000 and 17,000 yr BP, a range outside the quoted error for individual determinations, but within the realistic error in dating.

Fig. 2 Motion of the Stårnø virtual geomagnetic pole. Pole positions are computed from the sample inclinations (Fig. 1) and the interpolated values of declination from the long core measurement, corresponding to the age of each sample. Δ , Postglacial mean direction (7800 ± 100 BC).

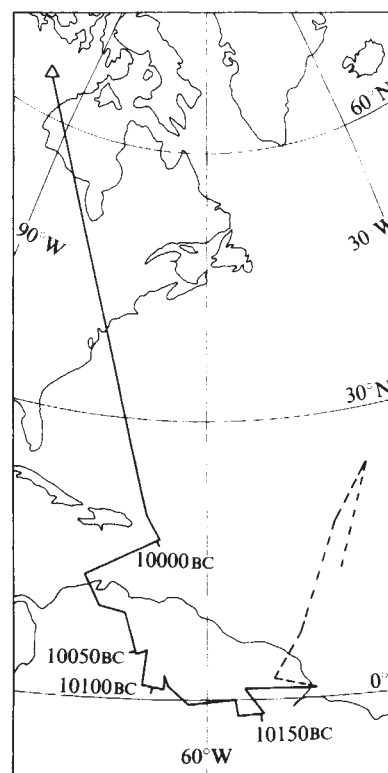


Table 1 Geomagnetic events probably corresponding to the Laschamp Event

Locality	Age (bc)	Dating method	Material	Reference
Puy de Laschamp, France	7000-9000	C ¹⁴	Volcanic scoria	15
Lake Tahoe, USA	≥ 1100		Lake muds	16
Gothenberg, Sweden	> 12350	C ¹⁴	Varved clay/silt	17, 18
Viby, Sweden	12400	Varve chronology	Varved clay	6
Lake Erie, USA	≥ 12500		Lake muds	19
Mono Lake, USA	< 13300	C ¹⁴	Lake muds	20
Lake Windermere, UK	13400 ± 400	C ¹⁴	Lake muds	21
Lake Chalco, Mexico	14450	C ¹⁴	Lake muds	22
Gulf of Mexico, USA	12500-17000	Fauna	Marine muds	23
Tlapacoya, Mexico	14770 ± 280	C ¹⁴	Lake muds	24
Gulf of Mexico, USA	17000 ± 1500	Fauna	Marine muds	25
Lake Biwa, Japan	17600-18700	C ¹⁴	Lake muds	26, 27
Laschamp + Olby, France	< 20000	K/Ar	Lavas	28
Blekinge, Sweden	12077-12103 ± 150	Varve chronology	Varved clay	This work

At least four other established excursions have occurred during the last 0.69 Myr: the Biwa II and I events at 295,000 and 181,000 BP, respectively²⁶, the Blake Event at 111,000 BP²⁹, and the Mungo Event at 30,000 BP³⁰. Others undoubtedly exist as such excursions are known throughout the geological record, for example during the Palaeo-Eocene³¹. Another event in silts of the Puget Lowland, USA was originally considered to be 20,000 yr old, but is now thought to be older than 45,000 BP. (K. L. Othberg, personal communication) and is, therefore, more likely to record the Mungo rather than the Laschamp Event.

In order to determine the age and duration of the Laschamp event we obtained samples of Swedish Quaternary laminated clays using the Swedish Geological Survey foil corer⁵, whereby undisturbed, oriented cores 3.6 cm in diameter and up to 11 m in length can be obtained. Varved sediments, which have not undergone bioturbation, are particularly suitable for palaeomagnetic investigations, and have been studied in Sweden^{6,7} and in North America⁸. Those studies involved measurements on individual samples, collected separately or extracted from cores.

After preliminary results had been obtained from a Swedish Geological Survey unoriented sediment core, new oriented cores were taken from two sites in southern Sweden (Blekinge), at Stilleryd (56.18° N, 14.83° E) and Stårnö (56.16° N, 14.85° E). Each core comprised a lower, varved, glacial sequence and an upper, postglacial, unvarved deposit. The susceptibility and natural remanence of each core were measured⁹ at intervals of 1 or 2 cm and then samples of characteristic lithology were selected from both sites to test their stability in alternating magnetic fields. The results indicated that the samples were moderately stable up to at least 600 oersted. The cores were then demagnetised in fields of 100 or 150 oersted. The remanence of the cores was interpolated to the positions of the inter-varve boundaries using cubic spline functions in order to adjust the data for changes in the rate of sedimentation. Age bounds for the postglacial sequences were fixed by relating the altitude of each site and its environs to the shorelevel displacement curve for eastern Blekinge¹⁰.

Each core was then cut into lengths of 5 or 6 cm and the samples were measured on a Digico magnetometer¹¹. The inclinations of remanence in the Stilleryd core which showed post-depositional chemical alteration of the sediment were found to be scattered, with no obvious trends. At Stårnö, the record of inclination has been preserved (Fig. 1), with a marked magnetic unconformity at the base of the postglacial deposit. The varves of that sequence have been correlated with those at Stilleryd and an absolute date has been determined by connection with a series in the Swedish chronology. This is generally considered¹² to be more accurate than radiocarbon dating and is probably correct to ± 150 yr (absolute) in the series considered here. The relative ages within each section are probably correct to within 5 yr. Measurements of the remanence immediately beneath two slumps in the Stilleryd core indicate that the magnetisation became blocked less than 9 yr after deposition and so the ages of sedimentation and magnetisation are virtually coincident in the undisturbed varve sequences.

We have computed the path of the virtual geomagnetic pole corresponding to the Stårnö palaeomagnetic record (Fig. 2). It indicates a geomagnetic quasi-reversal between 10153 BC

and 10127 BC followed by a northward migration of the virtual pole at a rate of about 2.8° of latitude every 100 yr. The southernmost excursion of the pole lies close to the meridian 60° W, 120° E, found by Steinhauser and Vincenz¹³ to be one of two preferred paths for palaeopoles during polarity transitions.

Studies of the unvarved sequences indicate anomalous changes in declination and low inclination values around 860 BC. This may well represent a more recent geomagnetic event, the Stårnö event, and may be the reversal reported by Ransom¹⁴ on the basis of archaeomagnetic studies by Folgheraiter and Mercanton on contemporary fired clay and pottery, from Greece and Bavaria. At this stage, however, the event is defined in non-varved sediments and we intend to examine the corresponding horizon within a varved sequence to determine the age and nature of this more recent excursion.

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Alternative to the geomagnetic self-reversing dynamo

A PRIMARY task of geomagnetism is to account for the magnetic field of the Earth and for the occasional reversals of that field. Present research in geomagnetic dynamo theory aims to develop a physically realistic, mathematically solvable, self-reversing dynamo model¹—one that contains the seeds of its own reversal. Here I point out that there is an alternative method of analysing the geomagnetic field which can account for magnetic reversals but which does not require a self-reversing dynamo. The analysis is based on the assumption that the geomagnetic field arises from two separate sources. Each source has a mathematical representation in terms of dipoles, quadrupoles, and so on. The magnetic field which is observed at or above the surface of the Earth is the sum of the fields arising from the two sources: in particular, the observed dipole component of the Earth's field is the vector sum of the dipole components of each source

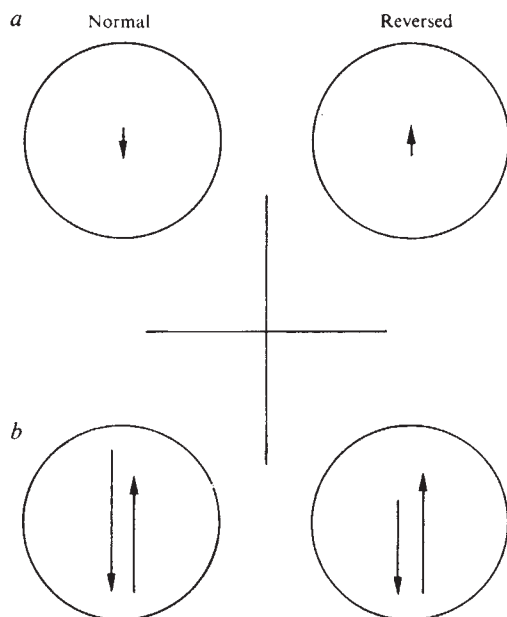
$$\mathbf{M}_0 = \mathbf{M}_1 + \mathbf{M}_2 \quad (1)$$

If the dipole components of each source are oppositely directed, one pointing essentially towards the north rotational pole and the other essentially towards the south rotational pole, then

$$\mathbf{M}_0 \simeq \mathbf{M}_1 - \mathbf{M}_2 \quad (2)$$

The prevailing magnetic polarity reflects the polarity of the dominant source component (Fig. 1). A magnetic reversal, which corresponds to a change in the sign of $\mathbf{M}_0$, represents

Fig. 1 Schematic diagram of polarity states. *a*, States of a self-reversing dynamo; *b*, states based on a two-source model. Net field is the same in both cases.



a shift in the relative sizes of $\mathbf{M}_1$ and $\mathbf{M}_2$. If both $\mathbf{M}_1$ and $\mathbf{M}_2$ are very large compared with $\mathbf{M}_0$, then only small fluctuations in $\mathbf{M}_1$, $\mathbf{M}_2$, or both will result in a reversal.

The foregoing analysis is appealing in that it avoids the complexities inherent in a self-reversing dynamo theory. First, a self reversal can not arise from a steady-state solution so that the full time-dependent solutions must be obtained for any given model. Rikitake² was able to demonstrate that a highly idealised double-disk dynamo showed time-dependent behaviour which resulted in self reversal. Subsequent work, however, has been severely hampered by the complexity of the magnetohydrodynamic fluid motions and the intractability of the associated equations. In the proposed model, a full time-dependent treatment is not necessary since one or both sources need only exhibit (small) fluctuations in magnitude when subjected to (small) perturbations.

Second, there is no need to treat the physical and thermal consequences which result from the large scale conversion of magnetic energy into kinetic energy and back to magnetic energy occurring during a self reversal³. But mathematical simplicity, in itself, is not admissible as evidence for a particular model; the observed data must be consistent with a two-source analysis and a plausible explanation for the two sources must be given.

If the Earth's field arises from two sources, then differences are to be expected between the normal and reversed polarity states, indicative of the fact that a different source is dominant in each state. Two such effects have been observed. Wilson⁴ grouped palaeomagnetic data by polarity and discovered significant differences in the time-averaged mean pole positions of the normal and reversed populations. Dagley and Lawley⁵ reviewed reported studies of transitional behaviour of the geomagnetic field and found a clear predominance of reversed over normal transitions, which led them to conclude that the reversed state is less stable than the normal state. A recent statistical study of the observed sequences of reversals by Phillips and Cox⁶ came to the same conclusion. Thus, systematic asymmetries may well exist between the two polarity states of the magnetic field.

Analysis of the present field may also reveal whether the Earth's field has two sources. I have developed⁷ a technique for fitting a set of spherical harmonic coefficients with a combination of geocentric axial dipole and eccentric (not necessarily axial) dipole. The free parameters are the relative strengths of the two dipoles as well as the coordinates of the eccentric dipole. When applied to the first eight coefficients of the International Geomagnetic Reference Field of 1965, the best fit consists not of two more or less parallel components whose sum is the present field, but rather two large, more or less anti-parallel components whose difference is the Earth's field (Fig. 2). This is precisely the configuration described in equation (2); so I conclude that there is some geomagnetic and palaeomagnetic evidence consistent with a two-source model.

As for an explanation of the two sources, the obvious possibilities are the inner and outer core. The source field from the outer core would still arise from a magnetohydrodynamic dynamo since the liquid nature of the outer core is well established. As noted above, steady-state solutions which exhibited fluctuations in magnitude would be adequate.

The central question is then whether the inner core can be the source of a magnetic field. That there exists a distinct inner core is now well established. The structure at the inner core–outer core interface has been mapped in considerable detail⁸. Recent observation of the propagation of shear waves through the inner core confirms that it is in a solid phase⁹. Clearly, a magnetohydrodynamic system is inappropriate for the generation of a magnetic field in the solid, inner core. A mechanism, if it exists, must come from solid state physics. A permanent magnetisation of the inner core resulting from ferromagnetism is not possible if the inner core is above its Curie temperature. Although this is probably the case, the theoretical extrapolations needed to conclude that the core is above its Curie temperature have never been tested experimentally at the pressures

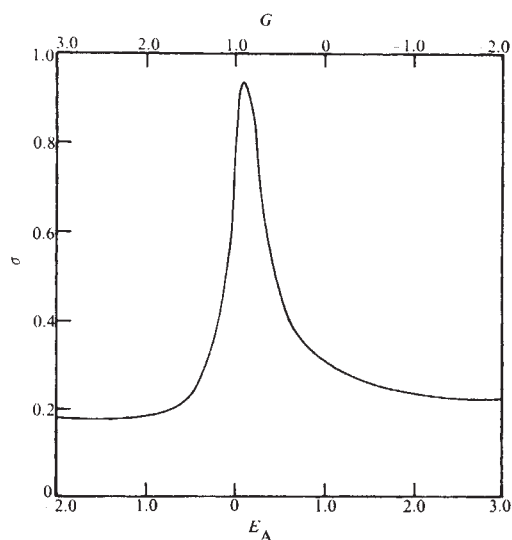


Fig. 2 Fit of the IGRF field with eccentric dipole and geocentric axial dipole. Parameter σ is mean square deviation in arbitrary units. E_A is the magnitude and direction of the axial component of the eccentric dipole. G is the magnitude and direction of the geocentric axial dipole. The vector sum of E_A and G is 1.0, the normalised, net axial component.

and temperatures of the inner core, so it is not known for certain whether the core is above its Curie temperature. Indeed, all thermodynamic parameters for the core must be based on extrapolation. Even the question of composition has not been completely resolved. In addition to iron, the inner core may contain large amounts of nickel and small amounts of carbon, sulphur and silicon¹⁰. Given this situation we can only conclude that the magnetic and electrical (semiconducting?) properties of the solid inner core have yet to be fully explored. So it is possible that at the particular and extreme pressures and temperatures of the core there may be new cooperative solid-state phenomena which could lead to a permanent magnetisation. Such a suggestion is not without precedent. In the case of hydrogen, Ashcroft¹¹ has shown theoretically that at high temperatures and pressures, superconductivity may occur.

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Is there an Icelandic mantle plume?

SCHILLING¹ has interpreted the chemistry of basalt lavas from the Reykjanes Ridge and Iceland, identifying two distinct upper mantle sources for these lavas, one of which rises in a primordial hot mantle plume beneath Iceland. I questioned² this interpretation on the grounds that insufficient attention had been given to the possibility that the magmas were derived from a homogeneous source; the differences could arise from by fractional crystallisation during the ascent of the lava.

Schilling³ pointed out two factual errors in my comments, which I accept. But his other comments reveal a misunderstanding of my ideas on the nature and effects of olivine-gabbro and eclogite fractionation. The facts of the distribution of the rare-earth elements (REE) as revealed by Schilling (ref. 1, Table 1), conflict with later statements³; they are, however, consistent with variable fractional crystallisation of lavas from a common source, or even from a source which is more depleted beneath the ridge.

Schilling's statements³ on primary magmas, the role of fractional crystallisation, and the significance of phenocrysts, seem to conflict with normally accepted models, and lead to constraints concerning upper mantle mineralogy and chemistry which were not among the many geophysical-geochemical requirements considered in setting up his model.

Basalts richest in REE do not show the depletion in the lighter REE which I formerly (incorrectly) expected² to result from significant fractionation of clinopyroxene (accompanied by olivine and plagioclase). There is now no impediment to hypotheses which involve clinopyroxene fractionation.

Schilling³ deduced that a high clinopyroxene-garnet ratio (~95:5) is necessary in any eclogite cumulus if the constraints of the rare earth element (REE) pattern are to be met. At a pressure of 30 kbar the coprecipitation ratio of clinopyroxene to garnet seems likely to be close to 57:43 (ref. 5), the same ratio at 40 kbar seems to be closer to 86:14 (ref. 6). The high clinopyroxene-garnet ratio in the extract is a result of the high solubility of Al_2O_3 in the pyroxene. Al_2O_3 , which at lower temperatures or other pressures would crystallise or exsolve as garnet, will be present as clinopyroxene in the actual cumulates which form from, and partition REE against, the liquid. The bulk extract still resembles the coexisting liquid in major chemistry, and massive extraction of an eclogite of this type would not necessarily lead to a major element chemistry distinct from that of the forerunners of the Reykjanes Peninsula olivine tholeiites (see ref. 5).

Schilling³ defined eclogite fractionation as "clinopyroxene-garnet extraction in a 50-50% mixture", implying that this ratio is required by O'Hara and Yoder's⁴ eclogite fractionation model: it is not.

Schilling³ challenged my statement² that there was massive relative depletion of heavy REE in the more northerly basalts. The La/Yb ratio is of the order of 0.28 in the more southerly basalts and of the order of 2.04 in the more northerly basalts. Relative to La, Yb in the more northerly basalts has been depleted to 0.14 of its abundance in the more southerly basalts; I consider that to be a massive relative depletion. The average Yb contents (from ref. 1, Table 1) of 13 samples taken south of 62°N are 3.0 p.p.m.; and those of 20 samples taken north of 63° 50'N are also 3.00 p.p.m.; for 9 samples taken north of 63° 50'N they average 2.0 p.p.m. There may, therefore, even be absolute depletion in Yb on passing north to Iceland, since the fall exceeds the $\pm 15\%$ precision quoted³. Schilling's (ref. 3, Fig. 1b) own graphical presentation demonstrates the validity of the very remarks which he himself says are wrong³.

I identified the upper drawing of Schilling's³ Fig. 1 as Fig. 1b and the lower drawing as Fig. 1a and I assumed that it is the basalts collected between 63°N and Iceland which are symbolised by the triangles.

In that case, Fig. 1b (ref. 3) demonstrates that the observed trend of $(Yb)_{EF}$ against $(La/Sm)_{EF}$ could be a result of eclogite fractionation at depth followed by olivine-gabbro fractionation near the surface, with the extent of both types of fractionation increasing northwards towards Iceland. This strongly supports my model in which "magmas rising through this thicker (Icelandic) superstructure . . . take much longer periods between formation and the eruption . . . allowing greater scope for fractional crystallisation at all pressures during ascent". Naturally, further south from 60°N, there will be little further change in $(La/Sm)_{EF}$ unless the thickness of volcanic superstructures and the consequent hydrostatic head of magma decreases markedly, which they do not so far as is known.

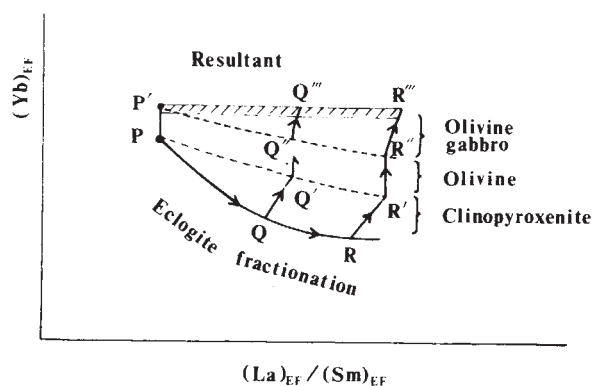


Fig. 1 Variation in the enrichment factor of Yb against the relative change in the enrichment factors of La and Sm during fractionation of REE. Vectors are proportional to the amount of fractionation undergone in different pressure regimes. The figure illustrates how the flat trend observed can be the result of the fractional crystallisation proposed. P, primary magma; P-P', fractionation path to erupted ridge magma; P-Q-R-R'-R''-R''', suggested fractionation path leading to Icelandic basalt.

Schilling³ now reports that $(La/Sm)_{EF}$ does not, in fact, decrease significantly on passing to the south along the ridge.

I have here considered only the effects of eclogite fractionation at high pressures and olivine gabbro fractionation at low pressures; but my model logically calls for fractionation in an intermediate pressure regime also, where crystal extracts are likely to be dominated by the separation of olivine and clinopyroxene (as in Yoder and Tilley's⁷ stage of pyroxenite fractionation). Figure 1 shows the nature of the proposed effects in the $(Yb)_{EF}$ against $(La/Sm)_{EF}$ plot. The possible influence of the participation of spinel in the crystal liquid equilibria should not be overlooked. Spinel could be expected to retain lighter REE.

Implicit in Fig. 1 is the concept that totally incompatible elements in erupted basalts may show enrichment factors (relative to their primary magma) ranging from 2 to 20, and differential enrichment factors as high as 10. Hart *et al.*⁸ clearly consider this to be unreasonable, but such a model is indicated by data for both the REE and the lithophile elements with large ions. Eclogite fractionation at high pressures does, moreover, afford the possibility that some K could be extracted (in omphacite) with Rb remaining excluded; that would lower the K-Rb ratio of the residual liquids (in accordance again with observed differences between Icelandic and ridge basalts).

The main source of our divergence of views may be the differing enthusiasm with which Schilling and I search for ways of explaining the minor and trace element chemistry; any explanations must, of course, be simultaneously compatible with current geodynamic hypotheses and with the major element chemistry which indicates that extensive fractional crystallisation is a common process in the history of erupted magmas. For example, changes in the nickel contents of basaltic liquids are controlled by the very high distribution coefficient for this element in favour of coexisting olivine and spinel crystals. But if all the rocks studied have suffered comparable fractionations of these minerals, there will be no wide divergence of Ni content. Eclogite fractionation, in contrast, has a much smaller effect on the Ni content of the residual liquids⁹. The combination of relatively small variations of Ni content with large variations in incompatible element concentrations, far from being an impediment to the proposed mode of eclogite fractionation⁸ is a fulfilment of another prediction for the eclogite fractionation model.

It is obvious from Schilling's¹ Table 1 that the large degree of differential fractional crystallisation implied by my Fig. 1 cannot be correct unless either; first, K_2O and P_2O_5 do not

behave as entirely incompatible elements (there is the possibility of loss of a fugitive phase from more fractionated basalts) or, second, the source mantle beneath Iceland is now significantly depleted in K_2O , P_2O_5 and perhaps $Fe/Fe+Mg$, relative to the mantle supplying the basalts at the ridge to the south of Iceland. The latter interpretation would be at least consistent with the observations that much more basalt has been produced in the past from the sub-Icelandic mantle. Although directly contrary to the primordial hot mantle plume hypothesis, this interpretation fits naturally into an alternative hypothesis suggested here.

Schilling referred¹ to "production of uniform primary magmas". "Primary magma" is a technical term which has been defined⁹ as a mass of magma which originated by tapping some body with a primitively liquid condition inherited from a remotely early stage in the Earth's history, or obtained by partial or complete fusion of pre-existing solid rock. That definition specifically excludes from the category of "primary magma" masses of magma modified by differentiation or contamination.

Schilling's model is specific and clearly stated^{1,4}. The sense in which Schilling³ now claims to have used the term 'primary' when discussing magmas differs from the text-book definition, which would have been confusing to igneous petrologists. Schilling's papers^{1,4}, however, show that he intended the term 'primary magmas' to be used in the same sense as that conveyed by the textbook definition.

In those papers^{1,4} the possibility of fractional crystallisation at shallow depths is discussed only in connection with its rejection as a mechanism for explaining the chemical variations, and as a cause of excess scatter over the dispersion error bars in a variation diagram (ref. 1, Fig. 2). Fractional crystallisation forms no part of the definitive statements of Schilling's^{1,4} model.

Rather than the model requiring fractional crystallisation near the surface to produce the observed phenocrysts, it is clear that Schilling envisages them as residual crystals from the partly melted source mantle, the mere presence of which in the lavas would necessarily exclude the possibility of fractional crystallisation.

If the phenocrysts are residual crystals from the partial melting of the respective source mantles at low pressure, four predictions would follow. First, the residual upper mantles would contain olivine, plagioclase and augite, but no orthopyroxene. Second, the fertile mantles would contain so little, if any, orthopyroxene that it would be the first major crystalline phase to be melted out during partial melting. Third, the residual mantles would contain olivines of composition Fo_{78-85} , and before melting the source mantles would have to be even less magnesian. Finally, the magmas from Iceland, derived from the primordial hot mantle plume would have to be in equilibrium with their phenocryst assemblage at a higher temperature than are the magmas from the ridge.

The first three predictions envisage an upper mantle quite different from popular models (such as those including nodules in kimberlite or basalt, orogenic peridotite masses, pyroclite (III)) in all of which orthopyroxene is prominent and remains with olivine as a residual crystal until very advanced levels of partial melting have been reached; and all of which envisage a considerably higher $Mg/Mg+Fe$ ratio in the source mantle, such that the olivines would have a composition Fo_{94-88} . The fourth prediction can be tested by experimental petrology but published data lead to the expectation² that it will be found to be untrue.

Schilling¹ has indeed listed and taken into consideration many geophysical-geochemical features in setting up his model. But among them I do not find included the geophysical consequences of low $Mg/Mg+Fe$ in the upper mantle, or the geochemical, indeed cosmochemical, consequences of lower Si/Mg and Mg/Ca ratios in the upper mantle than would be indicated from natural ultrabasic rocks or chondrite-based models of element abundances. Nor has he considered the

most fundamental geophysical objection to his hypothesis—density. Other models will have to be sought which fit all the constraints, not least among which is the fact that the erupted basalts are thoroughly fractionated at low and probably higher pressures, and cannot be used directly to characterise the geochemistry of their source regions.

Any mantle rock which is significantly more 'fertile' than another with regard to basalt production is going to be richer in garnet or spinel, and richer in Fe/Mg in its olivine and pyroxene, than less 'fertile' mantle. The more fertile mantle would therefore be appreciably denser than the less fertile mantle (by a factor of parts per hundred) and this difference could only be offset by a temperature difference of the order of thousands, rather than of tens¹, of degrees centigrade. (The orders of size used here are based on several considerations: the specific gravity of olivine increases by 0.014 g cm⁻³ for every 1 % decrease in forsterite content; if fertile mantle is the same as infertile mantle +20% potential basalt, and the potential basalt were crystallised as garnet plus pyroxene (density 3.6 g cm⁻³) distributed in the potential infertile peridotite residuum (3.3 g cm⁻³), the fertile mantle will have a density of ~2.5 parts per hundred higher than the infertile mantle. Coefficients of cubic expansion in silicates are taken to be ~10⁻⁵, not ~10⁻².)

In other words, if fertile mantle plumes exist at all, they should be sinking, not rising. It is, therefore, appropriate to consider at least two alternative models which are consistent with the relative densities of the rocks concerned.

The first retains the concepts of a plume of hot mantle material rising from great depths beneath Iceland, but supposes it to be composed of still fertile, yet more depleted mantle than is rising beneath the ridge (thus, it inverts Schilling's hypothesis). Its less fertile character would have been imposed by partial melting events which occur at the base of the upper mantle¹⁰. The resulting lower density of the partly depleted peridotite could cause it to begin to rise in localised diapirs through the more fertile and cooler overlying mantle. In spite of its less fertile character the rising diapir would nevertheless be capable of yielding abundant magma on account of its higher temperature and relatively rapid decompression. It is still necessary to suppose that magma eruption would be slower from this plume, because of the hydrostatic head, and that fractionation of the magmas occurs at all depths. An important factor would be the retention of at least some of the incompatible elements during the first partial melting events at the base of the upper mantle, caused by their incorporation in crystalline phases at very high pressures (~120–150 kbar).

An alternative to that hypothesis merely requires that a 'hot spot' starts because at some time there exists at some point a more rapid effusion of magma from the base of the upper mantle¹⁰. Following the build up of hydrostatic head in the magma once an island had been created, eclogite precipitation would begin and would lead to an increase in the density of the mantle beneath the 'hot spot'. That could lead to downsagging of the mantle beneath the 'hot spot' which in turn may be supposed to distort the stress field in such a way that the 'hot spot' continues to receive an undue share of magma from depth, thus becoming self propagating. This model does away with plume motion in the mantle and predicts a gravity high in the Iceland region.

Finally, I did not suggest that isotopes had been fractionated, but that isotope ratios had changed during the manifest fractional crystallisation. The distinction is vital.

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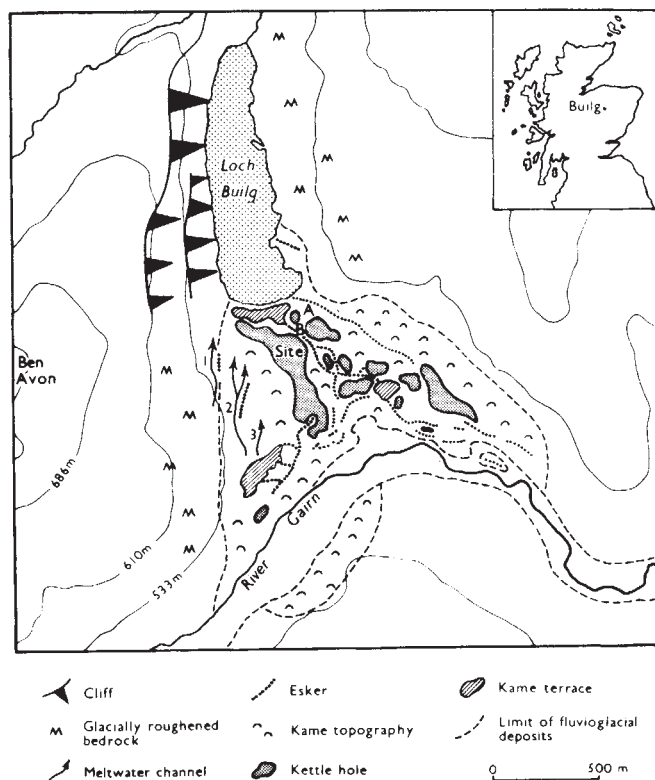
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Loch Lomond Readvance in the eastern Cairngorms

So far in the Cairngorm Mountains it has been impossible to confirm speculations about the physical extent of the glaciers of the Loch Lomond Readvance by absolute dating. As recently as 1970 it was therefore possible to argue without contradiction for anything between a full Scottish ice sheet and a handful of corrie glaciers¹. Subsequent evidence from the adjacent Spey Valley precludes the former possibility^{2,3}, and attention is now focused on the possibility that there was restricted ice cover about the time of the Loch Lomond Readvance. Evidence from the vicinity of Loch Builg, at an altitude of 525 m in the eastern Cairngorms, throws further light on the problem.

The main feature of this area is a marked concentration of hummocky landforms at the intersection of the River Gairn valley and the glacial watershed breach containing Loch Builg (Fig. 1). This topography is made up of steep-sided conical mounds, narrow ridges, flat-topped mounds and marshy or water-filled hollows. It has a relief amplitude of up to 20 m. The hummocks have previously been interpreted as the moraine limit of the Loch Lomond Readvance in this area^{4,5}. Field mapping strongly suggests, however, that they are a complex of kames and kettles associated with stagnant, downwasting ice of the last major ice sheet. There are few good sections exposing the internal composition of the hummocks but one of them does reveal well-sorted, crudely bedded sand and gravel and all

Fig. 1 Glaciated region around Loch Builg, Scotland.



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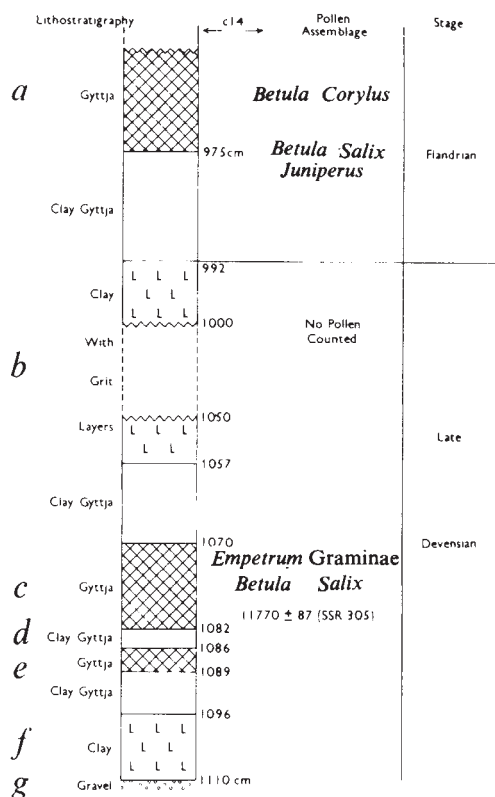


Fig. 2 Stratigraphy of the basal sediments at Loch Builg.

of the superficial exposures consist of water-worn pebbles and sand. Many of the hummocks have little obvious topographic alignment but numerous eskers and meltwater channels help establish the dominant trend and probable development of the former meltwater drainage system. There are two main directions of meltwater drainage indicated by the landforms. One enters the Loch Builg area from Glen Gairn and trends northwards into the River Avon valley; it is represented by channels 1-3 (Fig. 1) and contiguous eskers, all of which lie over 15 m above the valley floor and probably represent an early phase of drainage.

The other, lower group of eskers and associated kame terraces, shows the progressive realignment of the meltwater drainage system from the northern route to a lower, eastern route down the Gairn Valley. Eskers A and B (Fig. 1), for example, were probably deposited by streams that entered the western side of the Loch Builg breach from Glen Gairn, but curved back into the Gairn Valley when the route north had become abandoned.

The stratigraphy of an infilled kettle hole just south of Loch Builg (Ordnance Survey GR NJ 189028) has been examined using both a Hiller borer and, subsequently, a Jowsey borer. The basal sediments show features generally associated with the late Devensian stage. From 992-1,057 cm in the cores is a grey plastic clay (Fig. 2b) with stones and grit layers interpreted as a solifluction deposit. Beneath this, from 1,070-1,089 cm, is a brown organic gytja interpreted as an interstadial deposit, though there is a more complex stratigraphy than is usual, for a distinctly clayey layer (Fig. 2d) at 1,082-1,086 cm, divides the gytja into two parts (Fig. 2c and e). At the base is a grey clay (Fig. 2f) which gives way to gravel (Fig. 2g) at about 1,110 cm. This stratigraphy has been confirmed in several borings and there is no evidence for an ice advance over the site after the start of the deposition of these sediments.

Preliminary pollen counts of 200 grains have been carried out on samples collected by a Hiller borer from different depths in the sediments. Seven samples from gytja between

1,070 and 1,089 cm show an assemblage dominated by non-tree pollen. *Empetrum*, Graminae and Cyperaceae are most common, together with the shrubs *Salix* and *Juniperus* and open land herbs such as *Rumex*, *Artemisia*, Compositae, and Caryophyllaceae. Together, these make up 80% of the total pollen. The only tree pollen consistently present is *Betula* which comprises 10-20% of the total. This pollen assemblage is typical of the late Devensian in Scotland and similar assemblages have been recognised from the late Devensian at Abernethy³, the Allerod Interstadial at Loch Kinord, Loch of Park⁶, Garral Hill, Keith (where it has been radiocarbon dated between 10,800 and 11,880 b.p.)^{8,9} and elsewhere in northern Scotland¹⁰.

No pollen has been obtained from the clay between 992 and 1,057 cm.

In the uppermost gytja (Fig. 2a), sample counts have been carried out at widely spaced intervals. At 975 cm *Betula* pollen contributes 45% of the total land pollen, and *Juniperus* contributes 14%. *Salix*, *Empetrum* and Graminae are still important, but open land herbs have declined markedly. By 995 cm, *Betula* contributes 66% of the grains and *Corylus*, 13%. *Juniperus*, *Salix*, Graminae, *Empetrum* and the open land herbs have now greatly declined. At 900 cm *Betula* contributes 32% and *Corylus*, 58%. *Juniperus*, *Empetrum* and the open land herbs are no longer present. It seems that these pollen spectra compare well with the *Betula-Juniperus* Assemblage Zone and the *Betula-Corylus* Assemblage Zone of the early Flandrian at Abernethy³. They also compare with Zones IV and V at Loch Kinord, which again are early Flandrian⁶.

Thus, the pollen evidence suggests that the bottom deposit predates the Loch Lomond Readvance and that the clay deposit between 992 and 1,057 cm formed during the post-interstadial (Zone III) and is thus the time equivalent of the Loch Lomond Readvance.

Material for radiocarbon dating was obtained with the aid of repeated cores using a Jowsey borer. Gytja (Fig. 2c) was divided into an upper and lower half and bulked to form two samples: 1,070-1,067 cm and 1,076-1,082 cm. The bulked sample of the lower half of gytja (1,076-1,082 cm) is dated at 11,770 ± 87 b.p. (SSR 305). This date confirms both the interpretation of the stratigraphy and the pollen analysis: gytja (Fig. 2c and e) from 1,070-1,089 cm formed during the late Devensian interstadial and, therefore, predates the Loch Lomond Readvance.

It was not possible to obtain a sufficiently large sample of the lowest gytja (1,086-1,089 cm), (Fig. 2e) to allow radiocarbon dating, because gravel (Fig. 2g) at the base prevented penetration of the point of the corer. Certain suggestions regarding its age are, however, possible. If one assumes a constant rate of deposition and an even distribution of organic material in gytja (Fig. 2c), then a composite date for the lower half of the layer would represent a date of approximately one quarter the way through the period represented by the layer. As 11,770 b.p. is approximately one quarter of the way through the Allerod *sensu stricto* (10,750-11,950 bp)¹⁰ it is tempting to attribute the base of gytja (Fig. 2c) to a date of approximately 12,000 bp. Thus, it seems probable that clay-gytja and gytja (Fig. 2d and e) may be significantly older than 12,000 bp, though further dates are necessary to confirm or refute this. If this suggestion is confirmed then this site supports the evidence of early deglaciation in the Scottish Highlands which has already been suggested by radiocarbon dates and pollen stratigraphy from Wester Ross¹¹, the Spey Valley², the Teith Valley^{8,9}, Sutherland^{8,9} and Invernessshire^{8,9}.

The Loch Builg results have several implications for studies of Scottish Quaternary stratigraphy. First, the geomorphological evidence suggests that the fluvio-glacial landforms in the Loch Builg area are associated with the deglaciation of a major ice sheet and the only evidence in the vicinity for a later glacier readvance is in the corries on Ben Avon¹². This supports the interpretation previously suggested for the adjacent western Cairngorms¹.

Second, an area close to the heart of the Cairngorm Mountains, at an altitude of 525 m, was free of its main ice sheet cover earlier than 12,000 BP.

Third, the site has not been overrun by glaciers of the Loch Lomond Readvance. Thus, it is reasonable to suggest that any Loch Lomond glaciers must have been restricted to higher parts of the massif, for example the corries of Ben Avon.

Fourth, the identification of the limits of the Loch Lomond Readvance by mapping the distribution of 'fresh hummocky moraine' must now be considered to be a very precarious technique. The suggestion⁵ that the Builg hummocky moraine represents the limits of a Loch Lomond glacier in the valley of the Gairn is clearly contradicted by the evidence presented here. As argued elsewhere, many zones of hummocky moraine in Scotland may simply represent assemblages of types of stagnant ice-sheets at particularly favourable localities, usually determined by the shape of the underlying topography^{13,14}.

Finally, the apparently restricted distribution of Loch Lomond glaciers in the eastern Cairngorms contrasts markedly with the wide development of ice caps and outlet glaciers in the Grampians to the south and west^{4,5,8,9,15}. At present the contrast is difficult to explain.

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Distribution of foraminifera in surface waters of a coastal upwelling area

BIOCOENOSSES of planktonic foraminifera are usually accepted to be good indicators of different sorts of oceanic water masses¹, and it is assumed that their ecological requirements have not changed drastically during the Quaternary². Their remains have, therefore, been used for approximately the past 40 yr (ref. 3) to study, qualitatively as well as quantitatively, the palaeoclimatic record⁴ preserved in young, calcareous, deep-sea sediments. Despite this, however, the mechanism of the formation of death assemblages and sediment assemblages is very poorly understood, mainly because the distribution pattern of planktonic foraminiferal biocoenoses and their output of shell material is only poorly known.

Patchiness of faunas, differences between assemblages in different water masses, variability of hydrography over short time intervals and small distances, seasonal fluctuations of fertility and faunal compositions and changing turnover rates usually impede meaningful sampling. These difficulties are even greater in areas such as the continental margin of West Africa, where

Table 1 Varimax factor score matrix

	1	2	3	4
<i>Globigerinoides ruber</i>	0.292	0.001	−0.948	−0.008
<i>Globigerinoides sacculifer</i>	0.938	0.049	0.272	−0.026
<i>Globigerinoides tenellus</i>	0.035	0.021	0.005	0.006
<i>Globigerina bulloides</i>	−0.062	0.946	0.016	0.234
<i>Globigerina calida</i>	0.028	0.186	−0.021	0.218
<i>Globigerina falconensis</i>	0.001	0.034	0.008	0.017
<i>Globigerina quinqueloba</i>	0.085	0.103	0.103	0.641
<i>Globigerinella aequilateralis</i>	0.013	0.024	−0.003	0.010
<i>Globigerinita glutinata</i>	0.075	0.097	0.006	0.197
<i>Globoquadrina dutertrei</i>	0.097	0.153	0.117	0.550
<i>Globoquadrina pachyderma</i>	0.017	0.124	0.017	0.051
<i>Pulleniatina obliquiloculata</i>	−0.011	0.038	0.008	0.007
<i>Globorotalia inflata</i>	0.056	0.051	−0.016	0.310
<i>Globorotalia menardii</i>	0.051	−0.046	0.034	0.215

distinct water masses of the central gyres, of the eastern boundary-current system, and of the coastal upwelling zone, interweave in a complicated manner^{5–7}. A preliminary discussion of the planktonic foraminiferal biocoenoses living today in the surface water masses of that region is, however, desirable because of attempts to reconstruct the late Quaternary history of the area using planktonic foraminiferal taphocoenoses in sediments from the continental rise and slope off Spanish Sahara, Mauretania and Senegal⁸.

To minimise the difficulties in adequate sampling and to cover the area as thoroughly as possible, the near-surface water was sampled continuously during Cruise no. 25 of RV Meteor⁹. A pump supplied a set of filters with approximately 1.5 m³ seawater per hour from 4 m below the water surface⁹. Most samples contained 50–200 (maximum 500, >0.12 mm) planktonic foraminifera per cubic meter of filtered seawater. These values are higher than elsewhere in the Atlantic Ocean¹⁰, presumably because of the higher fertility of the surface waters in and around¹¹ the area surveyed.

The planktonic foraminiferal faunas consist of 26 different species⁹. As several of these showed a somewhat patchy and incoherent pattern of distribution, it was decided to reduce the number of variables by applying multivariate techniques¹². I have therefore applied Q-mode factor analysis to these samples using the Fortran-IV program CABFAC¹³.

The planktonic, foraminiferal biocoenoses found in the 98 samples of this study resolve into four important factors which together account for more than 90% of the total variance of the studied samples. At first, factor analysis was applied to all 26 species. The calculated factor scores indicate, however, that only 14 species (Table 1) are important contributors to the four factors.

Factor 1 (47% of the variance) is dominated by a group of species confined to the southern and western part of the area studied (Fig. 1a). The most important species within this factor is *Globigerinoides sacculifer*. This and the other significant species of Factor 1 dwell in tropical and subtropical, warm water masses¹. It is thus understandable that this factor shows a zone of minimum loadings along the continental margin of Africa, where a strip of cool water can be traced southwards almost to Cape Vert (Fig. 2).

Factor 2 (18% of the variance) which is dominated by *Globigerina bulloides* (Fig. 1b) has a distribution that is almost exactly opposite to Factor 1. The maximum loadings on this factor occur in the north of the area studied, in the region off Cape Blanc and Cape Barbas. A zone of higher values which can be followed southwards pinches out just north of Cape Vert. A similar distribution of *G. bulloides* in other plankton samples from the region off Cape Blanc has been described¹⁴. This pattern seems to be a rather persistent feature, as it has also been mapped in surface sediments^{2,3}, suggesting that it has existed for several hundred, if not several thousand, years.

The two remaining factors, (factor 3 accounting for 19% and factor 4 for 8% of the variance), also complement each other (Fig. 1c and d). Factor 3 is dominated by *Globigerinoides*

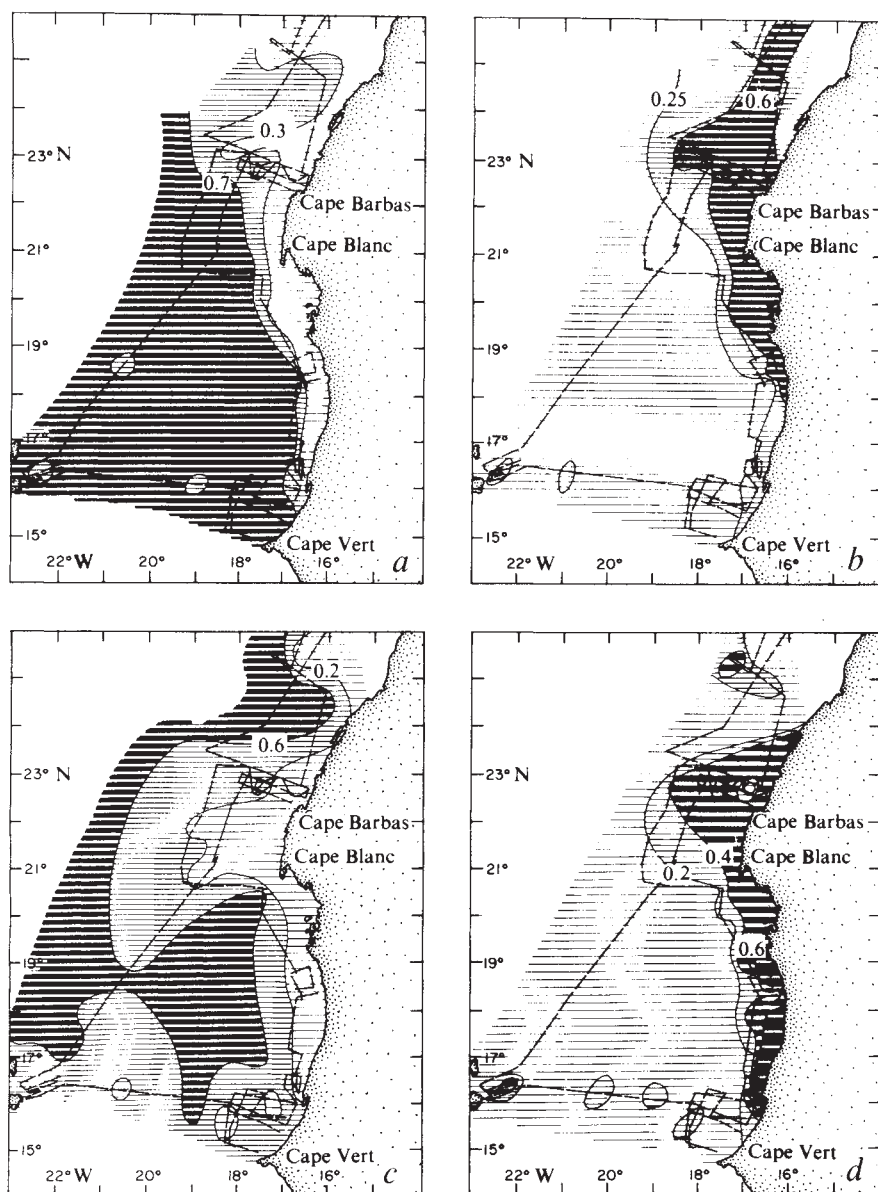


Fig. 1 Distribution of planktonic foraminiferal assemblages. Locations of pumping intervals corresponding to surface water plankton samples are marked by heavy lines along the course of the ship, RV Meteor cruise no. 25, October–December, 1971. Numbers, and associated shading and contours correspond to factor loadings, higher values indicating increasing abundance of respective foraminiferal assemblages. *a*, Factor 1, tropical warm water; *b*, factor 2, Canary Current, cool surface water; *c*, factor 3, tropical warm surface water; *d*, factor 4, cool upwelled water.

ruber. The regional distribution of this factor shows high loadings far from the coast in the central part of the region studied. That suggests that coastal upwelling has expelled normal surface water from the coastal zone. Thus, species such as *Globigerinoides ruber* which normally dwell in the uppermost layer of the water column are rarely found in the centre of the upwelling zone. If this interpretation is correct, the existence of another factor, caused by foraminiferal assemblages native to the upwelling water masses, could be expected; in fact, factor 4 seems to represent such an assemblage. Its highest loadings are found in the area where the factor 3 loadings are smallest.

Species dominating factor 4 are *Globigerina quinqueloba*, *Globoquadrina dutertrei*, and *Globorotalia inflata*. The highest loadings are found in the inner bight between Cape Vert and Cape Blanc, in samples which are distinctly green in colour because of their very high contents of photosynthetic organisms.

In my opinion, the distribution of this factor, the occurrence of abundant diatoms in all samples⁹ high in this factor and the fact that most of the foraminiferal species are subsurface dwellers¹, clearly indicate that factor 4 is representative of upwelling assemblages in this region. Factor 4 is sharply bounded to the north as well as to the south, and its seaward extension is very limited, the zone of highest loadings is confined to the innermost

few hundred meters. Its extent coincides well with the area of the most intensive coastal upwelling off West Africa¹¹. At first glance, it is puzzling that cool to transitional species, as well as tropical species such as *Globorotalia menardii* are grouped together in this factor (Table 1). In spite of the fact that the temperature of the surface waters are as much as 10° C lower in this region than in the open ocean (Fig. 2), *G. menardii* occurs in high absolute and relative abundances in a narrow belt which can be traced along the continental margin from Cape Vert to Cape Barbas, but not further north (Fig. 3). This distribution may be caused by a subsurface countercurrent flowing northward along the upper continental slope and outer shelf, presumably carrying *G. menardii* from a region further south where that species occurs most frequently at depth¹⁵. Upwelling of some of the undercurrent to the surface could then produce the anomalous distribution of the species in the studied area. Such an undercurrent is a common feature of upwelling regions^{5,16} and has been identified by deep current measurements⁷ off West Africa.

This pattern of distribution is geologically important, as *G. menardii* has been used widely in subtropical and tropical pelagic and hemipelagic sediments¹⁷ as a biostratigraphic indicator of warm climatic periods of the Pleistocene. Clearly,

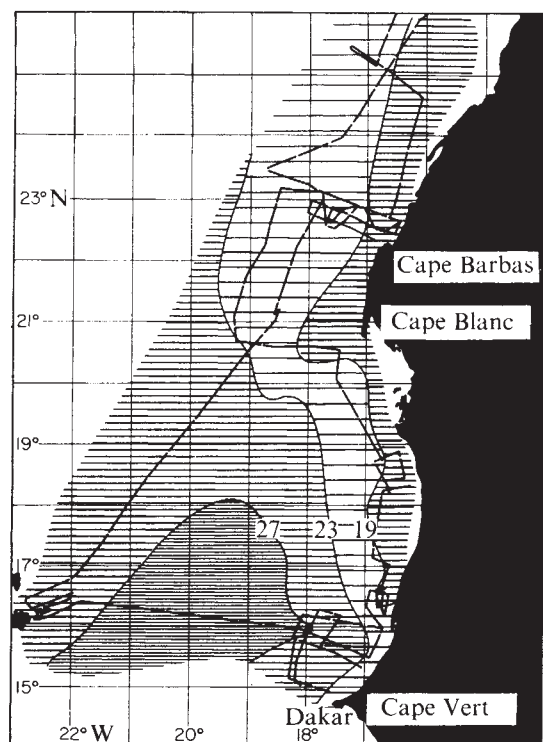


Fig. 2 Distribution of sea surface temperatures (°C) during late October to early December, 1971 off western Africa. Synoptic interpretation of daytime measurements corresponding to samples used for Fig. 1.

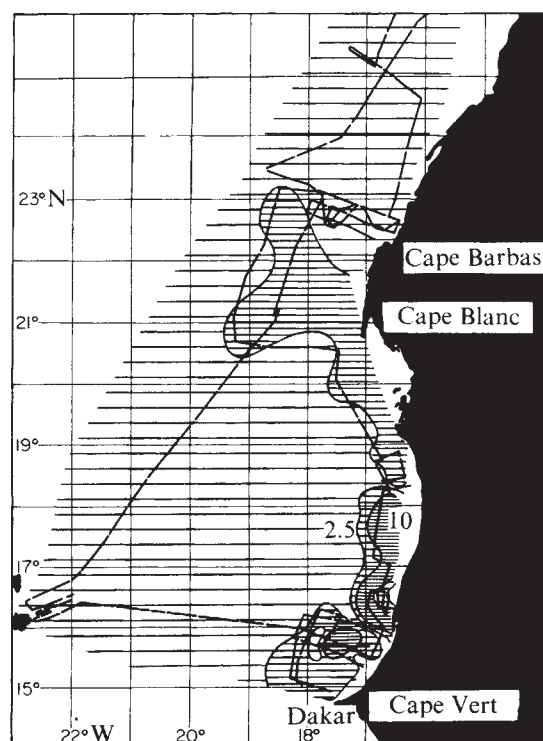


Fig. 3 Distribution of *Globorotalia menardii* (%) in surface water plankton samples.

the biostratigraphy of deep-sea sediments from upwelling areas must be re-evaluated in light of the possible problems revealed here. Thus, is it possible that intensified trade winds during glacial times trigger off much more intensive upwelling, and could that in turn intensify the subsurface countercurrent? If so, high abundances of *G. menardii* in sediments from likely areas may indicate only intense upwelling, thus presenting exactly the opposite picture from that usually proposed.

Another important point is that most of the species represented by the upwelling factor are highly resistant to dissolution, relative to other species. Although the pattern will be complicated by differing rates of turnover, the presence of the upwelling assemblages in the sediments may give the false impression that there is a heavily dissolved fauna at a depth close to the foraminiferal lysocline¹⁸. Although I suspect that the dissolution of calcium carbonate close to the continental margin is more intense than at comparable depths in the open ocean¹⁹, the data presented here do suggest that faunal indices which have been used to map the depth of the lysocline may be deceptive if the unusual character of the primary shell material is overlooked. Thus, the methods at present used to delineate the lysocline should be applied with caution, at least in upwelling regions, in coastal, and possibly also in equatorial areas²⁰.

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Recent cyclic changes in climate and in abundance of marine life

FLUCTUATIONS in temperature and rainfall are important to agriculture and human populations¹⁻⁶, and there is increasing interest in the possibility of forecasting future oscillations of climate on the basis of their apparent connection with more accurately predictable phenomena such as the sunspot cycle and planetary tidal cycles⁷⁻¹¹. A large part of the solar energy

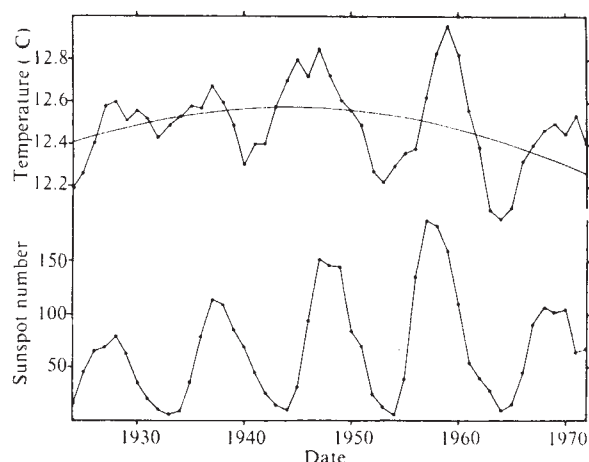


Fig. 1 Five-year running averages of sea surface temperature at station E1, compared with mean annual sunspot number (ref. 8 and personal communication from H. H. Lamb.) The secular trend of temperature is shown as a smooth curve obtained by fitting a polynomial regression.

budget of the Earth is mediated through the surface of the oceans¹², and it is well established that interaction between the sea, the sea currents and the atmosphere has important meteorological consequences and may also have direct or indirect effects on fisheries¹³⁻¹⁶. During the 1930s several attempts were made to link variations in solar radiation with biological and chemical events in the sea, such as the yield of certain North Atlantic fisheries and the quantity of algal nutrients present¹⁷⁻¹⁹. Tentative predictions made then were clearly ahead of their time, but the longer marine biological records now accumulating show signs of both secular and short period fluctuations which seem to be related in some way to corresponding cyclic fluctuations in sea temperature, and through these, to the solar and other cycles which have been

discussed recently in connection with terrestrial biological cycles^{6,5,20}.

We have analysed sea temperatures taken at International Hydrographic Station E1, situated in the English Channel some 20 miles south of Plymouth, and various biological measurements made in the vicinity. Mid-monthly temperatures and annual means were calculated from the monthly observations^{21,22}, and missing values for the war years have been calculated from mean differences from more continuous observations made inshore in Plymouth Sound^{23,22}; full details of this temperature analysis, including values for 70 m depth, will be given elsewhere. For present purposes the annual means of surface temperature at E1 were subjected to power spectrum (Fourier series) analysis and auto-correlation. Both methods showed the presence of major cycles of the order of 10-11 yr, but longer and shorter harmonics were also present. Filtering off short period variations by means of five-year running averages brings out the 10-11-yr cycle very well and illustrates the close agreement with curves of annual mean sunspot (Zurich) number (Fig. 1). Cross-correlation between the 11-yr component of the sunspot cycle and the annual mean temperatures gives an identical phase relationship; a similar phase agreement can be shown between the sunspot cycle and surface temperatures in the North Atlantic²⁴. The absence of any marked phase lag in the more inshore waters perhaps indicates a more direct link through atmospheric events rather than through changes in ocean currents. The short term variations, including the 11-yr cycle can be removed from the temperature records by calculation of a smooth curve (polynomial regression) to fit the annual means. The resulting secular trend is of much smaller amplitude than the shorter cycles but can be compared with the overall trend of the sunspot maxima.

The biological observations have been treated in the same way as the temperatures. Calculation of the 5-yr running averages indicates the presence of the same 11-yr cycle in such diverse data as the number of pilchard eggs in the plankton, the catch of demersal fishes and the proportion of barnacle species in the intertidal zone (Fig. 2). Removal of the secular trend from the biological data and annual temperatures, and cross correlation of the resulting short period fluctuations, shows significant correlation between temperature and several of the biological variables (Table 1).

Table 1 Biological data

		Cross correlation of temperature and short period trends (<i>r</i>)	Phase lag (yr)	Secular trend: linear regression against time (<i>r</i>)
Proportion of southern <i>Chthamalus</i> in the intertidal barnacle zone (1950-74)		0.78	2	-0.79
Catch per hour fishing on Plymouth grounds (RV Sarsia, 1954-72)	Hake Cod	0.50 -0.57	1 1	-0.61 (0.23)
Catch per hour fishing by trawlers landing at Newlyn, Cornwall (1948-61)	Hake Cod	(0.24) -0.69	2 1	-0.71 (0.44)
No. of pilchard eggs in a standard plankton haul at station L5 (Eddystone) (1947-74)	Summer Autumn	0.52 -0.56	-3* 1	(-0.35) 0.6
No. of non-clupeid postlarval young fish in a standard plankton haul at L5	1924-39 1957-74	(-0.35) (-0.26)	0-3 3-4	-0.80 0.87
Inorganic phosphate content of sea at station E1 in winter or early spring ('winter maximum')	1924-38 1948-74	(0.42) (0.28)	1 1	-0.82 (0.28)

Values in brackets indicate correlation not significant ($P > 0.05$).

*See text for explanation.

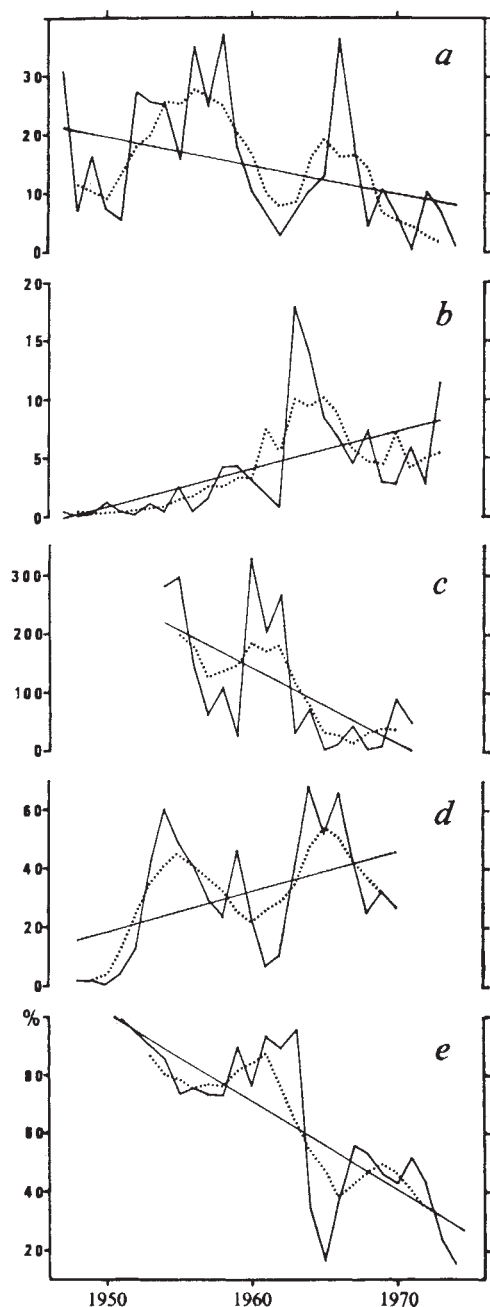


Fig. 2 Biological observations showing short period cycles and longer term trends. *a*, Number of pilchard eggs (thousands) in summer (sum of monthly means for April–July inclusive) at L5; *b*, number of pilchard eggs (thousands) at L5 in autumn (sum of monthly means for September–December inclusive); *c*, catch of hake (as number of fish per 100 h fishing) by RV Sarsia on the Plymouth grounds; *d*, cod landed by Newlyn trawlers (as hundredweights per 100 h fishing) from English Channel grounds; *e*, percentage numbers of *Chthamalus stellatus* (southern species) in total barnacle population on an intertidal traverse at the mouth of the Yealm Estuary near Plymouth. —, The annual values; . . ., the five year running average. The long term trend is shown as a linear regression.

Removal of the short period fluctuations from the biological data reveals the secular trend, and some of the values obtained are also given in the Table, as correlation coefficients of regression against time.

In general the warm water species (such as hake, red-mullet, John Dory, megrim, and the barnacle *Chthamalus*) are positively correlated with temperature and the cold water species (such

as cod, haddock and the barnacle *Balanus balanoides*) negatively so. In some the amplitude of the 11-yr cycle is small compared with the secular trend; for example, the proportion of *Chthamalus* in the total population of intertidal barnacles; the catch of hake, the numbers of young fish and the phosphate (algal nutrient) content of the sea before 1939. The secular trend seems to be less important than the shorter period fluctuations in the catch of cod, in the number of pilchard eggs in summer, in the phosphate content of the sea since 1948 and also in sea temperature. Pilchard are classed as warm water fish, the English Channel forming the northern limit of regular commercial exploitation. But the number of pilchard eggs in the autumn is negatively correlated with the 11-yr temperature cycle, and the secular trend is also negatively correlated with the secular temperature trend. This relationship has been discussed elsewhere²⁵ in connection with changes in the fishery and does not invalidate the general inferences drawn here. The summer pilchard eggs show a short period cycle which is in fact in advance of the temperature and sunspot cycle by 3 yr, so the correlation seems to be with the rapidly rising section of the curves rather than with the peaks. In some records, including a few not tabulated here, the long term trend is only vaguely connected with the general direction of the secular trend in temperature and there is little short term cyclic fluctuation; these particular examples, notably the numbers of postlarval young fish, the abundance of certain planktonic invertebrates and the phosphate content of the sea will be discussed fully elsewhere.

Some of the biological trends noted above have previously been found to be related to temperature by calculation of the regressions using the raw data, and a similar one to two year phase lag was shown^{26,27}. But the existence of the 11-yr cycle in these cases explains some of the obvious irregularities that had been found; some data not yet fully analysed suggest that short period cycles are widespread in marine biological and fishery observations, as for example in the catches of other species of fish in the North Sea and English Channel. The longer term trends reported here belong to a family of similar changes which have been found in several parts of the northern hemisphere and have been tentatively linked with the secular trend of sea temperature; these include fluctuations in arctic fishes and invertebrates, phytoplankton and zooplankton of the northern North Atlantic, North Sea fisheries and fish and invertebrates of the English Channel^{13, 28–33}.

The peak of the secular temperature cycle was reached in the 1940s or 1950s, and the trend has been in reverse since 1960 or earlier. If the suggested link between the biological trends, the temperature trends, and the solar and planetary cycles is correct, and if the predictions for the latter phenomena are correct^{7,11}, then the temperature trend should continue in reverse until 1990 or later, each successive 11-yr cycle bringing about a temporary re-reversal of lesser and lesser amplitude. On this basis it seems possible that we will see in the English Channel (and in other seas) further increases in the abundance of cold water species and decreases of warm water forms. It was remarked, concerning the rising phase of the secular trend, that the change in fauna had been detrimental to local English Channel fisheries³¹ (though beneficial in the Arctic²⁸), as many of the warm water species were of lesser commercial value than the cold water species they replaced. Some improvement to western English Channel fisheries has already taken place during the past 10 yr while the trend was in reverse, notably a return of cod and haddock and increases in mackerel shoals in certain areas, though there is a local belief that these effects are a benefit of the exclusive 12 mile coastal fishing zone recently claimed. In these days of closure of distant water northern fishing grounds and increasing fuel costs, a small improvement in near water fishing in the English Channel and adjacent seas may prove to be more beneficial than anticipated earlier.

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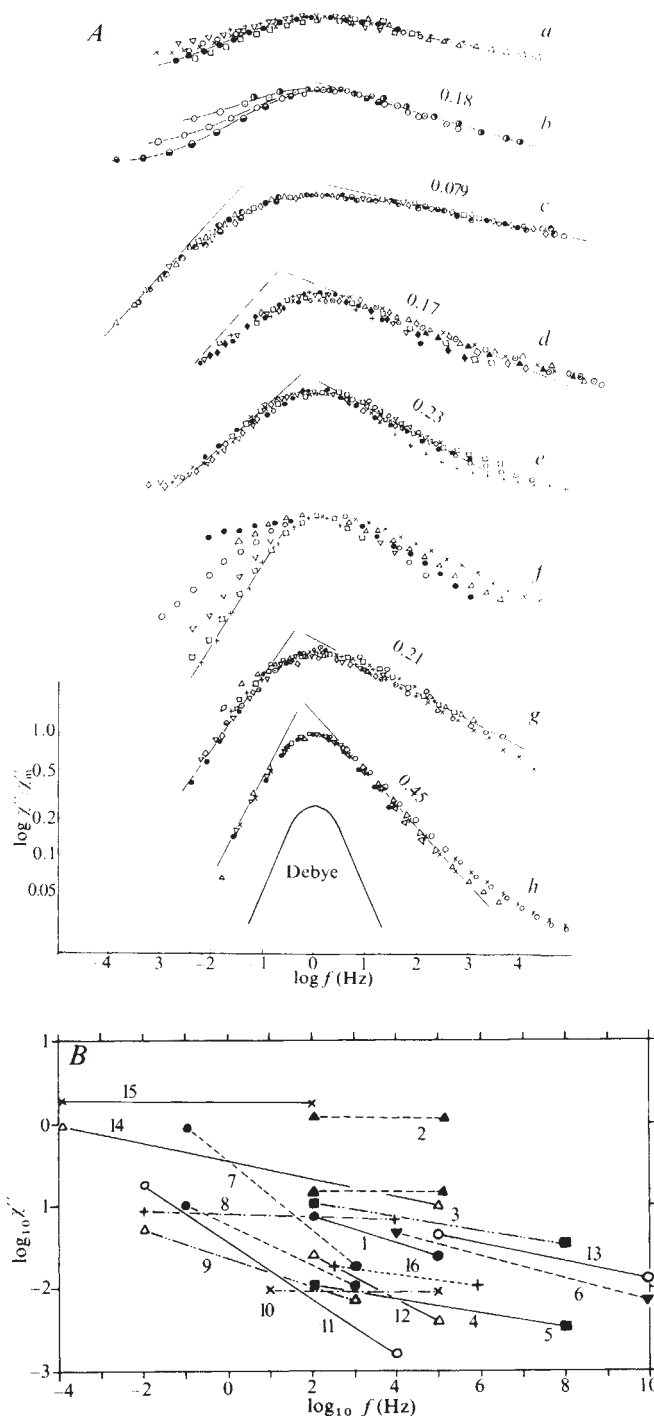


Fig. 1 A, A compilation of dielectric loss data for a range of polymeric materials². The points marked with different symbols correspond to data taken at different temperatures and transposed laterally to make the loss peaks coincide. The vertical scale is $\log f''$ and the consecutive graphs are displaced vertically for clarity. The numbers indicate slopes of the straight line portions of the graphs. a, Low temperature data for polychloroprene; b, PCTFE 12% crystalline; c, polyethylene terephthalate; d, polymethyl methacrylate; e, high temperature data for polychloroprene; f, polyethyl methacrylate; g, polydian carbonate; h, polyvinyl acetate. The shape of a pure Debye peak is also included for comparison. B, The corresponding compilation of loss data for a range of dielectric materials, including some amorphous inorganic solids, taken from ref. 4. Number code: 1, compensated single crystal silicon in the impurity hopping range; 2, $\text{Sb}_x\text{As}_{1-x}\text{S}_3$; 3, As_2Se_3 ; 4, As_2S_3 ; 5, Se; 6, As_2Se_3 ; 7, SiO_x range of temperature; 8, SiO_2 ; 9, Si_3N_4 ; 10, Al_2O_3 ; 11, Trinitrofluorenone-Polyvinylcarbazole; 12, solid polymerised CS_2 ; 13, Cu-Phthalocyanine; 14, Polyvinyl chloride, dioctyl phthalate; 15, Stearic acid; 16, Supercooled aqueous solution of LiCl.

Physical basis of dielectric loss

THE frequency dependence of the dielectric loss of all kinds of dielectric solids follows a 'universal law' ω^n , with $n < 1$ regardless of their physical and chemical nature. I show that, rather than being the result of superposition of Debye-like loss peaks, this is the manifestation of a universal mechanism for which the ratio of the energy lost per cycle to the energy stored per cycle is independent of frequency. A physical model is proposed which should apply generally to most dielectric materials and which has the required property.

The traditional approach to the interpretation of the frequency dependence of the dielectric loss is based on the Debye polarisation mechanism for which the complex dielectric susceptibility $\chi(\omega)$ is given by

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega) = [\epsilon(\omega) - \epsilon_\infty]/\epsilon_0 = (1 + i\omega\tau)^{-1} \quad (1)$$

where $\epsilon(\omega)$ is the complex dielectric permittivity at a frequency ω , ϵ_∞ is the limiting high-frequency value of ϵ , ϵ_0 is the permittivity of free space and τ is the dielectric relaxation time. This produces the familiar Debye loss peak at $\omega\tau = 1$ which is symmetric on a $\log \omega$ plot and has a half-width $\lambda = 1.144$ decades.

In general the loss does not obey equation (1) except in some liquid dielectrics and departs seriously from it in most solids. The commonly accepted interpretation of this invokes

a distribution of relaxation times, $g(\tau)$ such that the observed loss function is represented formally by the integral transform

$$\chi''(\omega) = \int_0^\infty g(\tau) \frac{\omega\tau}{1 + \omega^2\tau^2} d\tau \quad (2)$$

Examining the experimental evidence I conclude that equation (2) has little meaning but the observed frequency dependence of loss has a simple physical significance¹ and I propose a model which should be generally applicable. When the loss of many polymeric materials is plotted as $\log \chi''$ against $\log \omega$ with temperature as parameter, one obtains families of curves which may be 'normalised'² by shifting along the frequency axis (see Fig. 1) which gives a compilation of data for several materials. The loss may be represented by the empirical relation

$$1/\chi'' = (\omega/\omega_2)^{-m} + (\omega/\omega_1)^{(1-n)} \quad (3)$$

where $1/\omega_1$ and $1/\omega_2$ are thermally activated parameters with well defined activation energies. The exponents m and n are both smaller than one, and m is always greater than $(1-n)$ by a factor between 2 and 6 depending on the particular polymer and on temperature, resulting in a marked asymmetry of the peaks. Both m and $(1-n)$ tend to decrease with decreasing temperature resulting in very broad peaks having half-widths of 6 decades or more. The low-temperature β peaks are much broader and less symmetrical than the higher temperature α peaks³.

In addition to polymers, the dielectric loss in a wide range of inorganic materials in which the conduction is at least to some extent caused by hopping charge carriers¹ follows a law corresponding to the second term in equation (3) over a wide range of frequencies (ref. 4, Fig. 1B). But in these materials there is seldom a well defined loss peak at lower frequencies, instead the d.c. conductivity σ_0 dominates by contributing a term $\sigma_0/\epsilon_0\omega$. So I suggest that the dielectric loss in a very wide range of materials follows over a wide range of frequencies a 'universal law of loss'

$$\chi''(\omega) = B(T)\omega^{(n(T)-1)} \quad (4)$$

where $B(T)$ is a temperature dependent parameter.

The 'universal law' (4) cannot be plausibly explained in terms of $g(\tau)$ for many reasons; here I mention two of them. The physical difficulty of explaining the required range of τ values spanning not less than 8 to 10 decades, and the difficulty of understanding how the same power law (4) should result, albeit with different values of n , for most known solid dielectric materials, be they dipolar, electronic, ionic, organic, inorganic, polymeric, crystalline, amorphous, and so on.

Although the examples of Fig. 1 relate to selected simple cases which approximate to relations (3) or (4), I have shown (unpublished) that in many other cases where the loss exhibits a more complex behaviour it is possible to resolve the frequency dependence of both $\chi'(\omega)$ and $\chi''(\omega)$ into a superposition of two, or at most three, overlapping mechanisms of the type (3). I suggest that equation (3) is a more 'natural' function in which to 'expand' the experimentally observed loss data than the Debye function, which requires the assumption of a rather arbitrary distribution function $g(\tau)$. Using the universally applicable Kramers-Kronig relations, the empirically observed law (4) for $\chi''(\omega)$ implies the same frequency dependence for the real part $\chi'(\omega)$, so that the ratio χ''/χ' is independent of frequency¹. This should be contrasted with the Debye behaviour where this ratio is equal to $\omega\tau$. The consequence of this is that the ratio

$$\frac{W_l}{W_s} = \frac{\text{Energy lost per cycle}}{\text{Energy stored per cycle}} = \cotan\left(\frac{n\pi}{2}\right) = \text{constant} \quad (5)$$

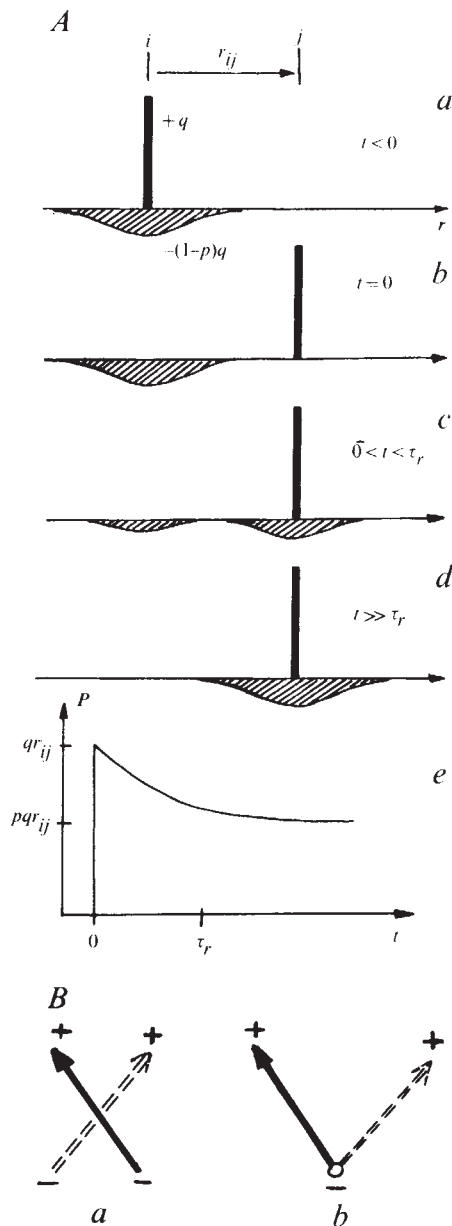


Fig. 2 A, The discontinuous jump of a charge q from a localised site i to a site j followed by a slower transfer of the screening charge. Diagrams a-d show consecutive stages, e shows the time dependence of the polarisation P resulting from the transition from i to j . B, The contrast between a 'free' dipole (a) and a 'pinned' dipole (b). A change of the orientation of the former does not bring about any change of the distribution of space charge, while a similar change for the latter does.

with respect to frequency. I suggest that this physically very simple criterion is the universally applicable common feature of all dielectric materials obeying equation (4). Conversely, if relation (5) is applicable, then the frequency law (4) is the only possible frequency dependence of loss.

What physical mechanism leads directly to the criterion (5)? I begin with materials in which there exist significant densities of localised electronic or ionic carriers capable of random hopping between spatially fixed centres. These materials often possess a significant d.c. conductivity and they are in many cases structurally strongly disordered. The presence of hopping conduction is an essential element of my present argument; free carrier conduction in conduction and valence bands lies outside its scope.

Consider now the hopping equivalent of a Debye dipole: a charge $+q$ which may hop between two localised sites i and j separated by a distance r_{ij} . The natural frequency of hopping between the two sites is $1/\tau_D$, where τ_D is a thermally activated Debye relaxation time. The loss resulting from this mechanism is given by equation (1) if no further reservations are made.

But our dielectric material contains a certain density of these localised charges, some of which may be able to hop over many consecutive sites ultimately giving rise to a d.c. conductivity σ_0 , while others may be restricted to shorter ranges, with the limiting case of pairs of hopping sites. A charge $+q$ localised on a site i imposes a Coulomb-like potential on the surrounding medium and this tends to repel like charges and to attract any oppositely charged particles that may be present in the neighbourhood, thus bringing about a partial screening of the charge in question. In the case of a gas of free carriers we obtain the screened Coulomb potential which implies that the effective charge decreases rapidly to zero outside a sphere of a few Debye lengths in diameter.

In a system consisting of localised charges the screening would not be as complete as that due to free charges, since localised charges are not quite free to assume the exact local density demanded by the local value of the potential. Nevertheless, we may postulate that the effective value of the charge q on site i is reduced to some smaller value pq , where $p < 1$.

If now the charge in question makes a rapid hopping transition to a neighbouring site j through a distance r_{ij} , the screening charge will initially be left behind on site i , so that the initial change of polarisation is $q r_{ij}$ and this only gradually decreases to $p q r_{ij}$ as the screening readjusts itself to the new position of the charge at j . I assume that this adjustment takes a time which may be loosely characterised by a relaxation time τ_r , without implying necessarily any particular form of time-dependence of this process. Assuming that the charge remains at site j for a time longer than τ_r (that $\tau_r < \tau_D$), the sequence of events involved in a hopping transition may be visualised with reference to Fig. 2A). The reduction of the resulting polarisation from $q r_{ij}$ to $p q r_{ij}$ corresponds to the reduction of the potential energy of the system discussed before¹ and the energy loss involved is dissipated through the phonon system.

The opposite case where $\tau_D \ll \tau_r$ is more likely to occur in nature and presents a qualitatively different picture, although the end result is the same. Since the screening space charge cannot follow the individual transitions, the screening charge adjusts itself to the time-averaged occupancies f_i and f_j of the two sites. The application of an external field E enhances the down-field rate and reduces the up-field rate resulting in a net change of occupancies by $\pm f'$ with respect to equilibrium. Each individual 'net' transition may be considered as the transfer of a 'bare' unscreened charge q through the distance r_{ij} ; but the subsequent readjustment of the screening charge gives rise to a final polarisation $P := p q r_{ij} f'$. Since all the net transitions take place in the field E , the energy derived from the field is $E q f' r_{ij}$ but the energy stored in the system after relaxation is only

$$W_s = E q p r_{ij} f' \quad (6)$$

and the energy lost is the difference between these two:

$$W_l = E q (1 - p) r_{ij} f' \quad (7)$$

I conclude that in either case, $\tau_r < \tau_D$ or $\tau_D < \tau_r$, the setting up of a given state of polarisation P in the system of hopping charges must give rise to a loss of energy which is proportional to the stored energy and which does not depend on the rate at which this state of polarisation has been established.

The energy loss W_l arising from the proposed screening mechanism is additional to any loss that may arise in an alternating electric field from the normal Debye delay τ_D in the response of hopping charges which gives rise to a loss given

by equation (1) and which may be considered negligible a decade or two below the frequency $1/\tau_D$. I am here concerned with losses extending down to much lower frequencies. The conditions which therefore have to be satisfied in a dielectric system for the universal criterion (5) to apply are: (1) discontinuous hopping of charges between localised positions, and (2) the presence of a screening charge adjusting slowly to the rapid hopping.

I have already stressed the point that the similarity of the functional dependence $\chi''(\omega)$ in hopping charge and in dipolar systems above the loss peak makes it plausible to look for essentially similar physical mechanisms of loss in both. How can this similarity arise despite many apparent differences? Dipoles in solids are not free to rotate as the idealised Debye model would require, but are constrained to assume discrete orientations in space with respect to the nearest neighbours, regardless of whether the material is crystalline or amorphous, since there is little difference between them in the short range order. Transitions between these discrete orientations occur abruptly in a manner completely analogous to the translational hopping of localised charge carriers, as required by condition (1) of my model.

An ideal dipolar system cannot give rise to screening, since no transfer of charge can occur. But all real molecular dipoles have finite length on an atomic scale and invariably one of their poles is more rigidly fixed in the 'lattice' than the other, as for example in the case of a polar side group on a rigid carbon chain in a polymer. I therefore introduce the concept of a 'pinned dipole', the response of which is not purely orientational but contains also an inseparable element of charge translation (Fig. 2B). Thus, pinned dipoles screen fields just as hopping charges do, but possibly less effectively.

Experimental results show clearly that the dielectric loss in most solids shows a simple power-law frequency dependence and this implies, as a consequence of Kramers-Kronig relationships, a frequency-independent ratio of energy lost per cycle to energy stored per cycle. I have suggested a model which satisfies this energy criterion and which is equally applicable to materials in which polarisation results from hopping of charge carriers and to those in which it results from dipolar molecular processes.

The proposed mechanism is much more plausible than the accepted interpretation in terms of a distribution of relaxation times. The physics of loss processes at and below the loss peak frequency will be the subject of a separate treatment.

The present work attempts to treat the dynamics of dielectric polarisation as a many-body interaction, by analogy with Calusius-Mosotti's static approach. The particular physical model proposed is not necessarily unique but the energy criterion (5) itself is well established by experimental facts.

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Electricity from photosensitisation of titanium

FUJISHIMA and Honda¹ have described a novel photochemical cell with an open circuit e.m.f. of up to 0.5 V which they indicated had a capability for water photolysis. The anode consisted of a TiO₂ n-type single crystal which was illuminated by light of wavelength shorter than 415 nm. This corresponds to the 3.0 eV band gap of TiO₂ (refs 2-4).

It has occurred to us that a photosensitive electrode for a photochemical cell might be formed equally well by oxidising a titanium electrode, and tests show that this seems to be the case. A very convenient way of making such a photosensitive surface is to pass a current between the Ti electrode and a platinum electrode both of which are immersed in an aqueous solution of 5 N KOH. The direction of current is such that the titanium electrode is the anode, and it consequently acquires an increasingly dark blue appearance as electrolysis continues.

In our experiment the oxidised titanium electrode (anode) and a platinum electrode (cathode) were mounted in the arms of a eudiometer to facilitate the measurement of gas uptake and evolution, with 5 N KOH as electrolyte. When the anode (fully immersed in electrolyte) is illuminated by a 100 W high pressure mercury lamp, and the cathode is partially exposed to air, oxygen is evolved on the illuminated face of the anode and current of the order of 1 mA cm⁻² flows. The open circuit voltage of the cell is approximately 1 V. The amount of exposed cathode area has no effect on these characteristics provided the length of the cathode-electrolyte-air interface is constant. But if the cathode is fully immersed in the electrolyte or if the air above it is replaced by nitrogen, the evolution of oxygen at the anode ceases and the current is greatly reduced.

So it seems, that oxygen is, under these conditions, adsorbed at the cathode-electrolyte interface; our measurements indicate that the volume of oxygen released at the illuminated anode surface is almost as much as the amount adsorbed. Gas evolution under these circumstances has not been observed at the cathode, but addition of an external e.m.f. of ~0.3 V results in the release of hydrogen at the cathode. This is consistent with the theoretical balance potential of 1.23 V. If a reduced TiO₂ single crystal is substituted for the titanium anode, the cell characteristics already noted remain essentially the same. We note that Boddy⁵ has also observed anodic evolution of oxygen at a rutile crystal-electrolyte interface.

The surface of titanium exposed to air develops a yellowish colour⁶ indicative of TiO. When a piece of untreated titanium is substituted for the electrolytically-treated titanium anode in the cell and connected to the cathode, a small current flows under illumination. Eventually it develops the same characteristics as the electrolytically-treated anode.

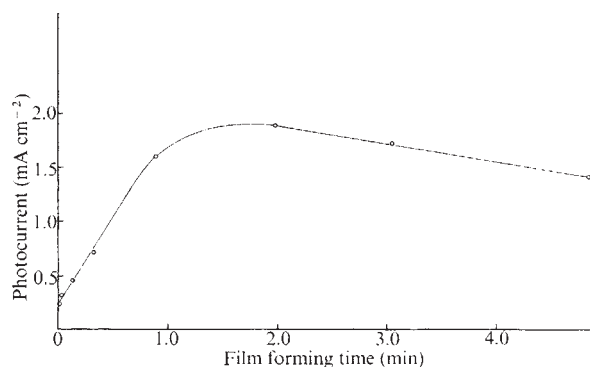


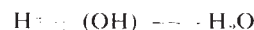
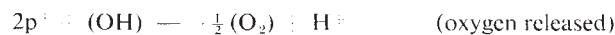
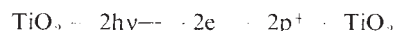
Fig. 1 Short circuit output photocurrent of oxidised titanium as a function of electrolytic forming time at 8 mA cm⁻².

The anode, which was formed electrolytically under constant current, was placed in the cell at intervals to determine the short circuit photocurrent capability (Fig. 1). There seems to be an optimum forming time.

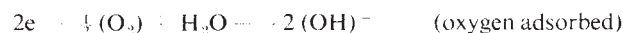
We have attempted to measure the corresponding weight increase of the titanium electrode and can establish an upper limit for the number of oxygen atoms as 10¹⁸ cm⁻².

If the concentration of KOH is reduced from 5 N to 1 N, production of oxygen is greatly reduced while the photocurrent remains substantially the same.

This suggests that more than one mechanism may be involved in our observations. One of these may be the following:



at the anode, and



at the cathode

Overall



Fujishima and Honda¹ have observed that the addition of more reducible species to the cathode compartment such as dissolved oxygen or Fe³⁺ serves to increase greatly the current flow. This leads us to believe that a scheme similar to that described above may play a part in their cell.

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Ionic conductivity in some bivalent metal sulphides

INVESTIGATIONS of the ionic conductivity of solids have been concentrated mainly on materials characterised by a purely ionic conductivity, such as AgI, RbAg₄I₅, and β-alumina, which have practical applications in the production of solid-state high energy-density batteries and other electrochemical devices¹. In such cases a non-zero electronic contribution prevents the material from acting as a solid electrolyte: materials with mixed ionic-electronic conductivity can be used as electrode materials in electrochemical cells.

A few materials (mainly oxides and sulphides) characterised by this type of conductivity have been studied. Investigations have been carried out on monovalent metal sulphides. Partial ionic conduction, dependent on stoichiometry, was detected by Hebb² in Ag₂S and by Hirahara³ in Cu₂S. Bivalent sulphides, however, have not been covered in the literature. But among these compounds are materials of considerable technological interest, such as ZnS. The existence of mixed ionic-electronic conductivity could be important to explanations of their behaviour as electronic devices.

By measuring the e.m.f. of suitable galvanic information cells¹, the ionic and electronic contributions to the electrical conductivity of some bivalent sulphides—such as the 2B group sulphides ZnS, CdS, HgS, PbS and CuS—have been determined.

We prepared samples comprising reagent grade chemicals. They were prepared from different raw materials and by different methods: precipitation and idrothermal synthesis. Impurity levels were determined spectrographically and were found to be 50–100 p.p.m. for ZnS and CdS (major impurities: Cu, Fe, Mg) and 50–150 p.p.m. for the other sulphides.

For each sulphide, the formation cell Me/MeS/S was established; Me consisted of a metal foil (a liquid Hg metal drop for HgS), MeS was a sulphide pellet (prepared by pressing the powder at 5 ton cm⁻²) which worked as the electrolyte, and the sulphur electrode consisted of rhombic sulphur, melted with 20% graphite powder and poured on the sulphide pellet.

The e.m.f. was measured at 25°C by means of a 10¹¹ ohm input impedance Keithley 630 potentiometric electrometer.

Table 1 Measured, E , and calculated, E^0 , e.m.f. of some galvanic cells

Cell	Crystallographic phase of MeS	E (V)	E^0 (V)
Zn/ZnS/S	Cubic	0.94 ± 0.01	0.98
	Hexagonal	0.83 ± 0.01	0.98
Cd/CdS/S	Cubic	0.69 ± 0.01	0.75
	Hexagonal	0.62 ± 0.01	0.75
Hg/HgS/S	Cubic	0	0.23
Cu/CuS/S	Hexagonal	0	0.25
Pb/PbS/S	Cubic	0	0.50

The average readings, E , are reported in Table 1 where they are compared with the E^0 values calculated from the standard ΔG^0 value of formation of the sulphide under examination⁵.

The reproducibility of E was found to be within ± 10 mV, using different samples with different impurity levels, prepared using different techniques.

Accepting certain assumptions¹, the e.m.f. of the cell can be expressed as a function of the sole transport number, t_e , of the electrons in the electrolyte:

$$E = (1 - t_e) E^0$$

and E^0 is in fact the value that E would achieve if the metal sulphide were to behave as a purely ionic conductor ($t_e = 0$).

The ionic transference number, t_i , of the sulphide may be obtained easily from the simple relationship

$$t_i = (1 - t_e) = E/E^0$$

where t_i represents the sum of all possible ionic contributions. In the case of the compounds considered here, however, t_i practically coincides with the transference number of the cation. The results are shown in Table 2, together with the

Table 2 Ionic transference number, t_i , and total conductivity, σ , of some metal sulphides

Sample	Crystallographic phase	σ (Ω cm) ⁻¹	t_i
ZnS	Cubic	3×10^{-7}	0.95 ± 0.01
	Hexagonal	10^{-6}	0.84 ± 0.01
CdS	Cubic	5×10^{-5}	0.92 ± 0.015
	Hexagonal	10^{-4}	0.83 ± 0.015
HgS	Cubic	5×10^{-1}	0
CuS	Hexagonal	5×10^{-1}	0
PbS	Cubic	10^{-2}	0

crystallographic phase and the total a.c. conductivity of the samples examined.

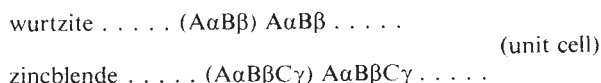
We found that zinc and cadmium sulphides are characterised by mixed conductivity, which has a significant ionic contribution. These sulphides have a very low total electrical conductivity which is strongly dependent on the frequency of the a.c. signal used, in the range 10²–10⁴ Hz.

On the other hand, in Cu, Hg and Pb sulphides, the contribution from the ionic conduction is practically zero. This group of sulphides is characterised by a higher total conductivity with negligible frequency effect.

Before we discuss these experimental results, we should first remark that, besides other factors, structural features have a decisive role in enhancing ionic conduction in solids.

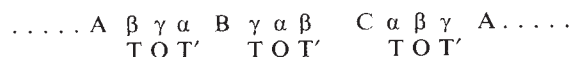
Availability of empty sites in the structure, where ions could jump from normally occupied sites, can be thought of as the essential condition before ions can significantly contribute to charge transport. That alone, however, does not seem sufficient in cases in which the existence of very narrow passage ways between occupied and empty sites should result in extremely high activation energies associated with the ion movement; other mechanisms would have to be operative. That seems to be the case with zinc and cadmium sulphides, as can be recognised by examining the ZnS structure in detail. This sulphide crystallises⁶ at low temperatures in the cubic zincblende form and at high temperatures in the hexagonal wurtzite form, with a transition temperature of $1,020 \pm 5^\circ$ C. A rhombohedral form and different polytypes also exist. The structures of zincblende and wurtzite are strictly correlated.

In both structures zinc and sulphur atoms are surrounded by four atoms of the other element at the apices of a regular tetrahedron. The second coordination sphere is very similar in the two structures; there is, however, some difference in the rhombohedral form. By considering the two structures along the close-packing direction (in this case zincblende is assumed to have a hexagonal cell) they can be schematised as:



where A, B, C and α , β , γ indicate the close-packing positions occupied by S and Zn, respectively.

In fact, three cationic layers—one with octahedral (O) and two with tetrahedral (T) sites—are available between the two sulphur layers. Only one of them is usually occupied (T'), as in the case of zincblende:



If the cationic and anionic sublattices are equivalent, this is also valid for sulphur atoms, but their contribution to ionic conduction can be questioned.

In an ideal structure, consideration of the ionic radii (Zn, 0.74 Å; S, 1.84 Å) shows that tetrahedral sites are very narrow and an atom is embedded at the centre of the tetrahedron; high activation energies can be expected for the ionic conductivity. The Zn–S bond is only partially ionic, thus atomic radii are nearer to tetrahedral covalent radii⁷ (Zn : 1.31 Å; S : 1.04 Å), but that does not vastly increase the freedom of movement.

The ionic conductivities of ZnS and isomorphous CdS at room temperature are orders of magnitude higher than those of alkali or alkali-earth halides at the same temperature. The presence of structural defects apart from empty sites and point defects, which enhance ion transport, can therefore be guessed at.

This seems to be particularly so in the sulphides discussed here, as they could contain domains of a second phase. As mentioned before, both phases contain empty sites, which

could be made accessible by stacking faults at the domain boundaries; so they could work as additional pathways for mobile ions. In fact, broken tetrahedral bonds are present to a large degree, because of the formation of stacking faults⁸. The not so different values obtained for the ionic transference numbers in zincblende and wurtzite phases (Table 2), are in agreement with the assumed model and can be justified because of the accuracy of measurement and because of variations of the total conductivity of samples.

On the other hand, in HgS we have not found any ionic contribution to conductivity. This compound, however, also exhibits two phases: cubic metacinnabar (isomorphous with zincblende) and trigonal cinnabar. The latter can be considered as a strongly distorted NaCl-type phase, without empty sites in the lattice. Thus, the absence of ionic conductivity in cubic HgS seems to emphasise the role played by two phases, both with empty sites and with slight mutual differences, in the contribution of ions to electrical conductivity, as we have observed in ZnS and CdS.

As for the other bivalent sulphides examined: PbS and CuS, they have only a crystallographic form, NaCl-type and hexagonal, respectively. Bearing in mind our previous considerations, their purely electronic conductivity is understandable. The enhanced electronic conductivity (with respect to ZnS and CdS) of those sulphides also reflects the structural differences, but we will not consider that here.

The presence of a mixed ionic-electronic conductivity in Zn and Cd sulphides holds a high technological interest in that such compounds are used in a number of electronic applications (electro and cathodo luminescent materials, photocells, and so on). As a matter of fact, the lifetime of these kinds of devices may be affected by ionic mobility, which could be considered as responsible for their ageing. Phenomena of this type are actually characteristic of the devices considered, and their behaviour should be reconsidered in the light of our results.

We thank Professor S. Pizzini for his suggestions and his interest in this work.

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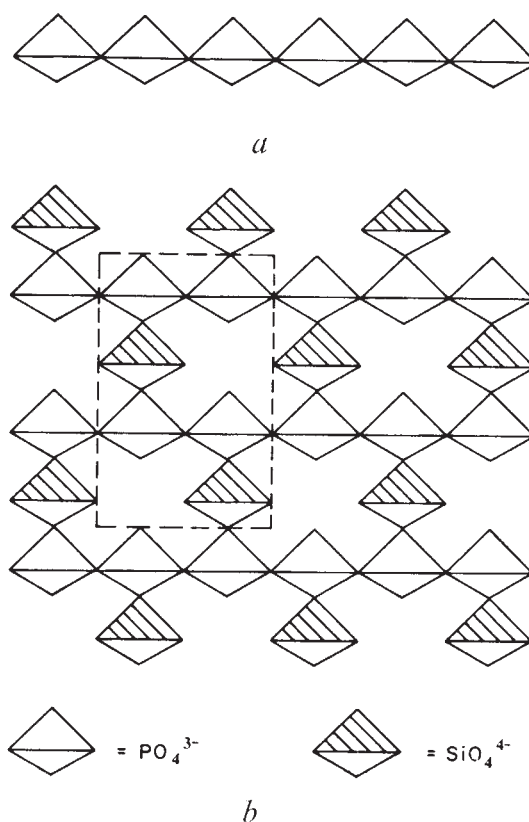


Fig. 1 One possible schematic representation of tetrahedra in the $P_2SiO_8^{2-}$ framework which may occur in $VO(P_2SiO_8)_x$. *a*, Shows a schematic metaphosphate $(PO_3)_\infty$ linear chain; *b*, shows how these chains might be linked by SiO_4 tetrahedra (the dotted line encloses a $P_4Si_2O_{16}^{4-}$ unit). No particular conformation of the 'layer' thus formed is implied. The 'layer' may also be derived from the one-dimensional pentaphosphate ribbon structure found²³ in NdP_5O_{14} , if the phosphorus in the cross-linking tetrahedra in the ribbons is replaced by silicon, and the ribbons are then linked together by silicate tetrahedra.

vite⁹ $[Na_5Ti_2(Si_2O_7)(PO_4)_2O_2]$ have recently been described. Natural phosphorus is mostly found in association with calcium in apatite minerals^{1,2}.

Using hydrothermal techniques, Barrer and Marshall² were unable to prepare viseite or any other material with substitution between Si and P, although many aluminosilicates and aluminophosphates were obtained; phosphorus substitution in several silicate zeolite frameworks was achieved by Flanigen and Grose¹⁰ using a hydrothermal gel crystallisation technique. Otherwise, except for the chemical analysis of minerals such as viseite and wilkeite, few systematic studies of phosphosilicates have been made, and this limited attention is no doubt the result of their infrequent occurrence in nature. The reason for this lack of Si(IV)-P(V) substitution is not entirely clear, but may reflect the lack of appropriate geological preparative conditions, which allowed the formation of relatively more stable species such as the apatites, rather than any fundamental instability.

Many possible formulae for phosphosilicates may be derived, however, and new crystal structures with perhaps interesting properties may be envisaged. We report here the preparation and some properties of oxovanadium (IV) diphosphatomonosilicate, $(VO)^{2+}(P_2SiO_8)$. The stoichiometry of this material may indicate a novel arrangement of condensed tetrahedra, and the ease of preparation indicates that phosphosilicates may indeed be a potentially extensive class of materials.

Small (up to 1 mm) blue-green crystals of $VO(P_2SiO_8)_x$ were prepared by vapour transport in a quartz tube using iodine as the transporting agent and $VO(PO_3)_2$ as the starting material.

Novel phosphosilicate

Few studies of materials containing both phosphate and silicate tetrahedra have been reported. This may seem surprising as large numbers of natural or synthetic aluminosilicates and aluminophosphates are known^{1,2}. But the substitution in minerals of P(V) for Si(IV) in silicate structures, or of Si(IV) for P(V) in phosphate structures is not common. Among the minerals described to have any significant degree of P(V)-Si(IV) partial substitution are viseite³, nagatelite⁴ and wilkeite⁵, an apatite with partial $SiO_4 + SO_4$ substitution for PO_4 . Some synthetic phosphosilicates, chiefly apatites, are described in refs 6 and 7. A very few phosphosilicates where Si and P may be crystallographically independent are also known, and the structures of silicocarnotite⁸ $[Ca_5(PO_4)_2SiO_4]$ and lomonoso-

(VO(PO₃)₂ was prepared by reduction of V₂O₅ by hot phosphoric acid. Its properties, and those of V³⁺(PO₃)₃, prepared in a similar fashion, will be described elsewhere.) Transport was obtained with the hot zone at 1,100 °C. X-ray emission analysis of a single crystal indicated the probable stoichiometry and this was confirmed by preparation of polycrystalline material by solid state reaction. 1:1 molar pre-dried VO(PO₃)₂ and SiO₂ were heated for 2 d at 1,040 °C in a sealed platinum tube. The X-ray powder pattern of the polycrystalline material was identical to that of a crushed single crystal, and quite different from those of the starting materials. The polycrystalline preparation was analysed for total vanadium, phosphorus and silicon by the Schwarzkopf Microanalytical Laboratory, Woodside, New York 11377. The calculated proportions by weight were: V, 17.9%; P, 21.7%; Si, 9.9%. The proportions found were: V, 17.9%; P, 22.0%; Si, 9.5%. The incorporation of silicon into crystals grown on the tube walls from the vapour is not uncommon in experiments carried out in quartz tubes. For example, the preparations of Ba₂Nb₁₄SiO₄₇ (ref. 11) and Yb₃(SiO₄)₂Cl (ref. 12) have been recently described. Precession photographs of a single crystal (Mo K α radiation) of VO(P₂SiO₈) indicated the tetragonal space group P4/n.c.c. (No. 130) with cell dimensions $a_0 = 8.697$ (8) Å and $c_0 = 8.119$ (8) Å. Conoscopic viewing of the optic figure confirmed the crystal to be uniaxial. The measured density (2.9 ± 0.1 g cm⁻³) indicates four molecules per unit cell.

In common with VO(PO₃)₂ and V(PO₃)₃, VO(P₂SiO₈) may be heated to at least 1,000 °C in air without oxidation or decomposition, and is insoluble in most non-oxidising solvents. These unusual characteristics for reduced vanadium compounds entailed the characterisation of the vanadium species by magnetic and spectral methods rather than by chemical analysis of the oxidation state. The magnetic susceptibility was measured on a polycrystalline sample between 1.5 K and room temperature using a pendulum magnetometer. Curie-Weiss behaviour was observed between 300 K and 10 K with $\mu_{\text{eff}} = 1.70\mu_B$, which is characteristic¹³ of VO²⁺ compounds (3d¹). The paramagnetic Curie temperature is 0 ± 1 K but antiferromagnetic ordering is observed below 2.5 K. Magnetic ordering is not frequently observed in d¹ compounds ($S = \frac{1}{2}$), but β -VOSO₄ is reported¹⁴ to be antiferromagnetic below 25 K and α -VOSO₄ ferrimagnetic below 4 K. Also, VF₄ is a canted antiferromagnet below 28 K (ref. 15). The diffuse transmission electronic spectrum of polycrystalline VO(P₂SiO₈) shows three bands centred at 12,500 cm⁻¹, 15,250 cm⁻¹ and 27,000 cm⁻¹, characteristic of V⁴⁺ species containing a distorted VO₆⁸⁻ octahedron^{13,16} and close to the positions of the bands in VO(PO₃)₂. The infrared transmission spectrum (KBr disk) reveals a band at 951 cm⁻¹ attributable to V-O stretching and other bands at 782, 807, 1,030, 1,113, 1,162, 1,230, 1,260 and 1,325 cm⁻¹. Bands at 682 cm⁻¹ and 710 cm⁻¹ observed¹⁷ in SiP₂O₇ and which are characteristic of octahedrally coordinated silicon¹⁸ are not observed here.

As is found in most tetravalent vanadium compounds, the V⁴⁺ ion in VO(P₂SiO₈) seems to be surrounded by an octahedron of oxygen atoms, but displaced towards one oxygen with the formation of one short and one long V-O bond. In VOSO₄·5H₂O (ref. 19) and VOSO₄·3H₂O (ref. 20) and many vanadyl complexes¹², the VO₆ octahedra are isolated from one another, with the nearest neighbour oxygen bound only to the central vanadium and the remaining five oxygens being provided by the other groups present. In α - and β -VOSO₄ (refs 14, 21) and VOMoO₄ (ref. 22), the VO₆⁸⁻ octahedra are linked in infinite chains by the alternating long and short V-O bonds, with the four equatorial oxygens of each octahedron being provided by the XO₄ groups. The presence of magnetic ordering in VO(P₂SiO₈) indicates that the VO₆⁸⁻ octahedra cannot be totally isolated from each other, but the lower vanadium content relative to VOSO₄ and VOMoO₄ may entail a somewhat different arrangement of octahedra than is found in these compounds. It is perhaps surprising that magnetic ordering occurs at all in VOSO₄ and VO(P₂SiO₈), for there is only one unpaired electron per mag-

netic atom, and even in VOSO₄ which has the higher vanadium content, the VO₆⁸⁻ octahedra are directly linked in only one dimension. VO(PO₃)₂ which seems to have a structure containing isolated chains of corner linked VO₆⁸⁻ octahedra like VOSO₄, shows no evidence of magnetic ordering down to 1.5 K.

The formula of VO(P₂SiO₈) indicates that isolated PO₄³⁻ or SiO₄⁴⁻ ions are very unlikely to be present in the structure, and this material seems, therefore, to be the first reported condensed stoichiometric phosphosilicate. The stoichiometry (X₃O₈) of condensed tetrahedra is also unusual and we are not aware of the existence of any analogous silicate, M(IV)Si₃O₈, or phosphate, M(I)P₃O₈. One possible way of linking the tetrahedra in such a situation which could also provide a basis for ordering of P and Si, is shown schematically in Fig. 1.

The ease of preparation of VO(P₂SiO₈) indicates that condensed phosphosilicates may be a more extensive class of materials than has been previously suspected. The preparation of other vanadium species, as well as phosphosilicates of other metals may be anticipated. New types of condensed tetrahedral framework may be formed, perhaps permitting the ability to tailor materials to obtain desirable properties.

We thank Professors R. M. Barrer and D. McConnell for discussions and Dr W. R. Robinson for pointing out the preparation of phosphosilicate zeolites. K. L. Tai assisted with the X-ray emission analysis and G. A. Pasteur measured the spectra. *Note added in proof:* C. M. Barrer and M. Liguornik²⁴ were unable to reproduce the results of Flanigen and Grose¹⁰ concerning the hydrothermal preparation of phosphosilicate zeolites.

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Measurement of tensile strength of liquids by an explosion technique

A WIDE variety of methods has been used in the estimation of the static tensile strength of water^{1,2}. Recently, a dynamic method, using an impulse-generated pressure wave, has been described which uses a measurement of the negative pressure produced by the wave reflected from a free surface³. Wilson *et al.*⁴ had previously shown how estimates of the negative-pressure wave reflecting from a free surface could be used to measure liquid tensile strength in underwater-explosion research, and an adaption of that technique has been used to



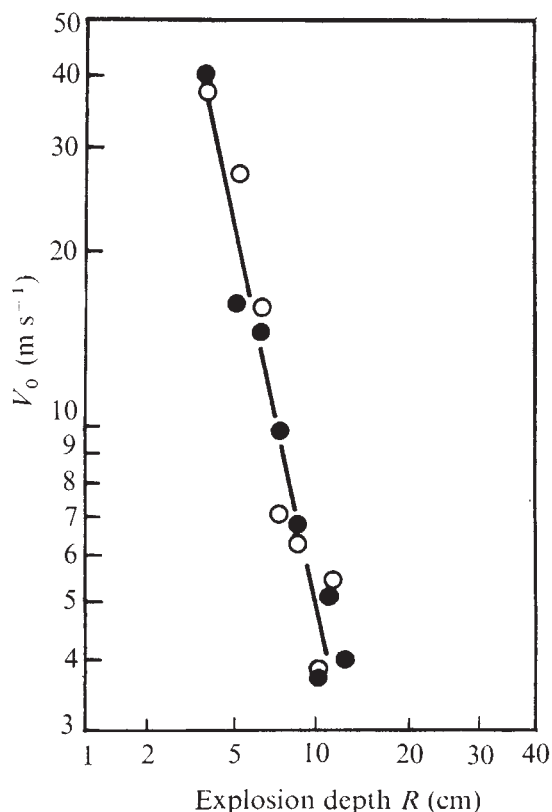
Fig. 1 Experimental set up for measuring liquid tensile strength by explosion technique.

estimate the tensile strength of water and glycerine. The basic idea is to make high speed motion picture photographs of the spray plume rising from an explosive charge detonated a small distance underwater. In a one-dimensional analysis the particle velocity of the explosive shock wave propagates as $P/\rho U$, and hence water at the free surface detaches with an initial velocity $2P/\rho U$, less the contribution of the tension exerted by the reflected shock wave $T/\rho U$. So the initial spray dome velocity is:

$$V_0 = (2/\rho U)(P - T/2)$$

where P is the maximum pressure of the explosion, T is the negative pressure in the reflected shock wave, ρ is the water density,

Fig. 2 Measured spray-dome velocity as a function of explosion depth. ●, Water; ○, glycerine.



and U is the shock wave velocity. By observing the value of V_0 at various explosive charge depths and extrapolating to zero velocity where $T = 2P$, an estimate of the water tensile strength can be made.

The experimental equipment is shown in Fig. 1, where the latter stage of a test is pictured. A small electrically actuated detonator containing 0.1 g of explosive was placed at depths from 2.5 to 12.7 cm below the surface in the centre of an open oil drum, filled to the top with the liquid under study. The only other equipment required is a high speed motion picture camera; we used a Photo Sonics camera at a speed of 500 frames s^{-1} . The displacement of the initial spray dome was measured from the film using an optical comparator. Typically, five or six frames per test contained the initial spray-dome rise data.

Figure 2 shows the results of the spray-dome velocity measurements as a function of charge depth, R . Extrapolation to the limit of measurement accuracy, 0.2 m s^{-1} , gives a value of R (for $V_0 \sim 0$) of 31 cm.

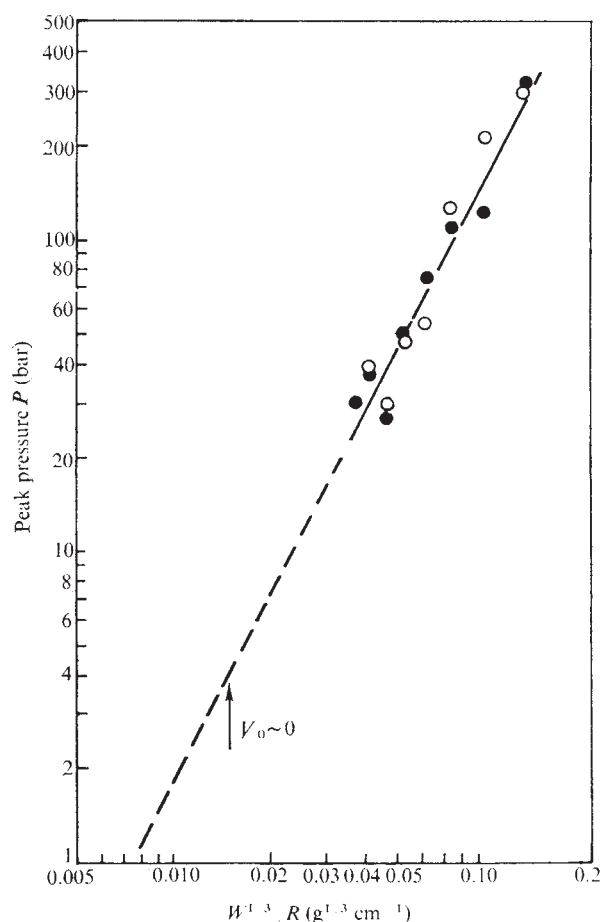


Fig. 3 Peak pressure as a function of explosive similarity parameter. ●, Water; ○, glycerine.

Figure 3 gives the peak pressure data plotted against the similarity parameter⁵ for explosives, $W^{1/3}/R$, where W is the charge weight and R the depth of the charge. The peak pressure, P , was obtained from the particle velocity through tables⁵ of the Rankine-Hugoniot equations. Using the extrapolated value of $W^{1/3}/R$ corresponding to $V_0 = 0$, an estimate of P and thus $T/2$ can be formed. We obtained a value of $T = 8 \text{ bar}$, in good agreement with the result of Couzens and Trevena³, who obtained a value of 8.5 bar by direct measurement of the reflected wave. Both water and glycerine gave the same results.

This relatively simple way of estimating liquid tensile strengths may find application in comparative measurement of fluids such

as drag-reducing polymer solutions which cannot be subjected to the elaborate purification requirements of more conventional methods of measuring tensile strengths of liquids.

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Adaptive foci in protein evolution

THE *willistoni* group of *Drosophila* consists of several species, subspecies and semispecies endemic to the New World tropics¹. We have used electrophoretic techniques to study allelic variation at gene loci coding for enzymes in this group of species. Some 40 gene loci have been assayed in scores of natural populations belonging to 14 different taxa²⁻¹¹. Two remarkable patterns have emerged. First, within a given taxon the populations (sometimes geographically separated by thousands of kilometres) have quite similar configurations of allelic frequencies at nearly all loci; and second, the allelic configurations observed in different taxa show that any two species have quite similar genetic configurations at about half the loci, but very different configurations at (nearly) the other half. A similar situation obtains when infraspecific taxa are compared, except that the proportion of loci at which the two taxa are similar is more than half, and the proportion of loci at which they are totally different becomes much less than half.

The sets of loci at which any two taxa have similar genetic constitutions are different when different pairs of taxa are compared. At any one locus we find groups of two or more taxa genetically very similar to each other, but very different from the other groups; but the taxa that are similar at, say, locus A, may be different at locus B, whereas taxa that are different at locus A may be similar at locus

C, and so on. At most loci, two, three, or four different genetic configurations are found, with two or more taxa fitting each configuration.

We have suggested¹¹ that in the *willistoni* group of *Drosophila* the allelic frequencies cluster around a few orthogonally located 'adaptive peaks', or 'adaptive foci'. We also argued that this clustering could not be explained by the particular evolutionary history of the taxa (founder effects and bottlenecks in population size, phylogenetic relationships, and so on), as it is often the case that closely related taxa are in the same focus for some enzyme loci, but in different foci for other loci at which some of the taxa may share foci with less closely related species. Using the method of principal component analysis, we can provide support for both contentions.

Our analysis (Table 1) deals with seven species, six of which are morphologically almost indistinguishable. Two species consist each of two geographically separated subspecies; a third species consists of six semispecies or incipient species. In each of these 14 taxa we have analysed the allelic frequencies at the same 21 enzyme loci. The average number of genomes studied per locus is 15,036 for the whole group, or 1,074 per taxon. For each locus we have performed a principal component analysis in which taxa were taken as variables and allelic frequencies as cases. Geometrically, principal component analysis rotates Cartesian axes, maintaining orthogonality, in such a way that fewer axes are needed to explain the experimental variance. In our case, the original axes are frequencies of single alleles; the rotated axes are polymorphic combinations of the original axes (an unrotated axis remains monomorphic).

Table 1 shows, for each taxon at each locus, the single principal component which explains most of the experimental variance. There is a marked tendency for taxa to lie almost parallel to other taxa, so that usually several taxa lie on the same principal component. At the *Lap-5* locus, for example, seven taxa share principal component 1, four taxa share component 2, two taxa share component 3, and one taxon lies on component 4. The table indicates the proportion of the experimental variance explained by the principal components given. At the *Lap-5* locus, 98.3% of the experimental variance is explained by four principal components. Over all 21 loci, the average proportion of the experimental variance explained per locus is 95.0%. This is accomplished with an average of 2.9 principal components

Table 1 Principal components and proportion of variance explained for each of 21 gene loci in 14 taxa of the *D. willistoni* group. The mean number of alleles per locus is 12.8 ± 0.8

Loci	Taxa*														Percent explained of total variance
	<i>t</i>	<i>ww</i>	<i>wq</i>	<i>ee</i>	<i>ec</i>	AM	IN	OR	AN	CA	T	<i>pv</i>	<i>i</i>	<i>n</i>	
<i>Lap-5</i>	2	2	2	1	1	1	1	3	1	1	1	3	4	2	98.3
<i>Est-2</i>	1	2	1	2	1	1	1	1	1	1	1	3	4	3	97.0
<i>Est-4</i>	2	2	1	1	3	1	1	1	1	1	4	1	5	3	99.9
<i>Est-5</i>	2	1	1	1	3	1	1	1	1	1	1	1	2	2	99.9
<i>Est-7</i>	2	2	5	3	3	1	1	1	1	1	1	3	3	4	85.3
<i>Acp-1</i>	4	3	3	2	4	1	1	2	1	1	1	2	1	1	89.4
<i>Ald-1</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.0
<i>Adh</i>	3	1	1	1	1	1	1	1	1	1	1	1	1	2	99.9
<i>Mdh-2</i>	3	2	2	1	4	1	1	1	1	1	1	1	3	4	86.9
<i>α-Gpdh</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	98.0
<i>Idh</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99.6
<i>Me-2</i>	1	1	1	1	1	1	2	2	1	1	1	2	1	3	89.5
<i>Xdh</i>	3	1	3	1	1	1	1	1	1	1	1	1	2	2	77.3
<i>To</i>	1	1	1	1	1	1	1	1	1	1	1	1	2	2	92.0
<i>Tpi-2</i>	1	1	1	2	2	1	1	1	1	1	1	1	1	3	99.9
<i>Pgm-1</i>	2	1	1	2	2	1	1	1	1	1	1	1	2	3	99.9
<i>Adk-1</i>	1	1	1	1	1	1	1	1	1	1	1	1	2	3	99.3
<i>Adk-2</i>	1	1	1	2	2	1	1	1	1	1	1	1	2	2	99.5
<i>Hk-1</i>	2	2	2	2	3	1	1	1	1	1	1	1	3	1	90.1
<i>Hk-2</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	98.0
<i>Hk-3</i>	1	1	1	1	1	2	2	2	1	1	1	1	3	1	99.8

* Symbols for the taxa: *t* = *D. tropicalis*; *ww* = *D. willistoni willistoni*; *wq* = *D.w. quechua*; *ee* = *D. equinoxialis equinoxialis*; *ec* = *D.e. caribbensis*. *D. paulistorum* semispecies: AM = Amazonian; IN = Interior; OR = Orinocan; AN = Andean; CA = Centroamerican; T = Transitional; *pv* = *D. pavlovskiana*, *i* = *D. insularis*, *n* = *D. nebulosa*.

per locus. The original dimensionality (the number of axes or alleles) ranges from 8 to 21 with an average of 12.8 per locus. Table 1 also shows that the taxa that share principal components are different when different loci are compared, although more closely related taxa (like the six semispecies of *D. paulistorum*) tend to share principal components at more loci than less closely related taxa.

Our results indicate that, at each locus, natural selection favours one of a few alternative genetic configurations. With an average of 13 alleles per locus, we have a 13-dimensional genetic space for each locus. Yet all taxa fall within a few small foci (on average three) orthogonal to each other. These foci seem to be maintained by selection, whereas other combinations of alleles are not. Species evolve by changing their allelic frequencies so as to move rapidly from one adaptive focus to another, as evidenced by the fact that intermediate configurations rarely occur¹¹. Why the observed combinations of allelic frequencies are favoured in the *willistoni* group of *Drosophila* is a question for which we have no answer. It seems likely that selection may act on these loci as members of coadapted multilocus systems. Particular configurations of allelic frequencies occurring at some loci may affect the allelic configurations favourably selected at some other loci.

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Pre-Columbian purslane (*Portulaca oleracea* L.) in the New World

LIKE so many cosmopolitan weeds, purslane (*Portulaca oleracea* L.) has a history characterised by paradox and uncertainty. Most botanists have followed De Candolle¹ in assuming that it is native to the Old World and was introduced in the New World. There is, in fact, convincing historical evidence that the species was present in Europe before 1492 (ref. 2), and furthermore that it was introduced to North America in the seventeenth century³. On the other hand, the historical record also contains several anomalously early references to a wild purslane in the New World, and some botanists have argued that the species may have been present on both sides of the Atlantic before 1492 (ref. 4). Asa Gray even went so far as to suggest that the Vikings may have introduced it during their occupation of Greenland and Newfoundland⁵. We now present conclusive evidence that purslane was present in the New World in pre-Columbian times.

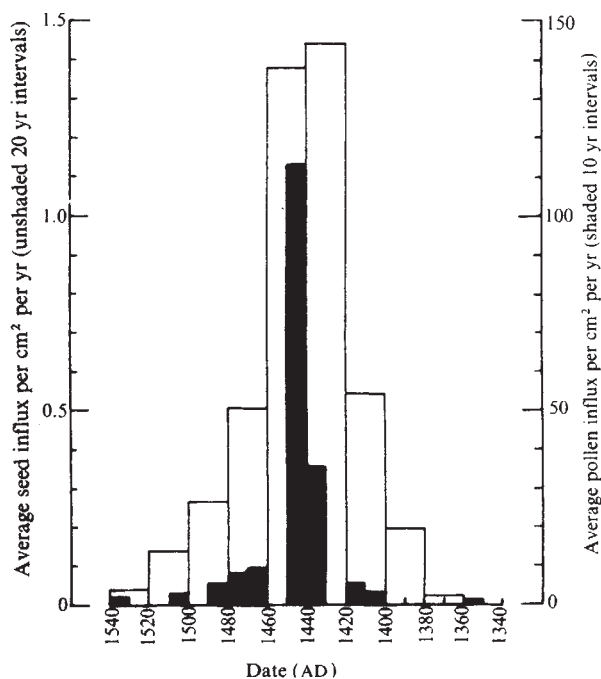
Purslane pollen and seeds were found in the sediments of Crawford Lake, which is some 35 km south-west of Toronto, Ontario. It has a surface area of 2.4 ha and a maximum

depth of 24 m. Largely because of its morphometry, there is an incomplete circulation in the water column (meromixis) and consequent anoxia in the bottom waters. The sediments are therefore free from disturbance by either water currents or both organisms. The sediments are varved, each varve consisting of a white summer layer rich in carbonate and a dark, winter layer rich in organic matter. Similar sediments have been described from other lakes in Ontario and adjacent New York State⁶⁻⁷. The significance of the varves in the present context is that they can be used to establish an accurate sediment chronology for the past 700 yr.

Sediment samples were obtained from the deepest part of the lake by freezing them *in situ* onto the outside surface of a 2-m long aluminium tube loaded with dry ice. The field and laboratory techniques used in sampling laminated sediments have been discussed in more detail by Swain⁸. Routine pollen analysis of contiguous 10-yr intervals indicated that purslane pollen was deposited in the lake during the period 1430-89 AD. More intensive analysis extended the range from 1350 to 1539 AD but not to the present. Figure 1 shows the pollen influx in absolute terms. The total pollen influx (arboreal pollen AP and non-arboreal pollen, NAP) into Crawford Lake during this period was approximately 3,500 grains per cm² per yr. Purslane pollen is relatively large and has a distinctive pericarpate arrangement of furrows (Fig. 2). It cannot be confused with any other pollen type in the local flora⁹. The identification was further confirmed by the discovery of purslane seeds in sediments from the same period. Ten samples (2.5 cm³) from contiguous 20-yr intervals were sieved through a No. 50 mesh (hole size 297 µm) and the residue was analysed for macrofossils. The seeds are generally well preserved, and are characterised by stellate epidermal cells. They correspond closely with modern Ontario herbarium material (Fig. 3). In terms of influx rates they show a similar if somewhat more regular trend than that of the pollen (Fig. 1).

The discovery of purslane pollen and seeds was unexpected. Apart from the problem of supposed Old World origins, the question arose as to how the fossils were deposited into the lake. Pollen dispersal through the atmosphere seemed unlikely as purslane is primarily a self-pollinating species¹⁰. Furthermore, the seeds, although small,

Fig. 1 Average pollen and seed influx.



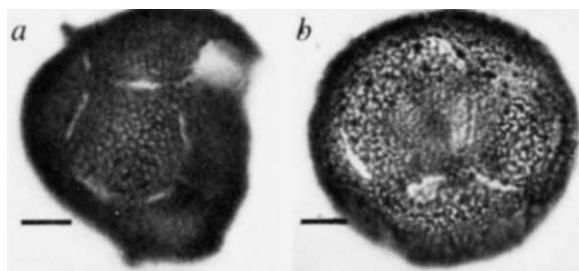


Fig. 2 Photomicrographs of *Portulaca oleracea* pollen grains. *a*, Fossil pollen grain (slightly broken) from Crawford Lake sediment dating 1430-39 AD. *b*, Modern pollen grain from southern Ontario. Scale bars represent 10 µm.

are not well adapted to dispersal by wind. Water transport also seemed unlikely as the lake is fed by a spring. The mechanism proposed here is that the pollen and seeds were deposited in the lake by man. More specifically, that agricultural Indians, who lived in the immediate vicinity of the lake, gathered purslane in their corn fields and washed it in the lake before eating it as a potherb. This washing would not only remove the soil from the plant but also release the pollen and seeds into the lake. That agricultural Indians were in the area at this time is also indicated by the presence of maize pollen (*Zea mays*) and sunflower pollen and seeds (*Helianthus annuus*) in the sediments dating from 1360 to 1650 AD. The fossils did in fact lead to the discovery of an Indian village site less than 1 km from the lake. The site is currently being excavated¹¹.

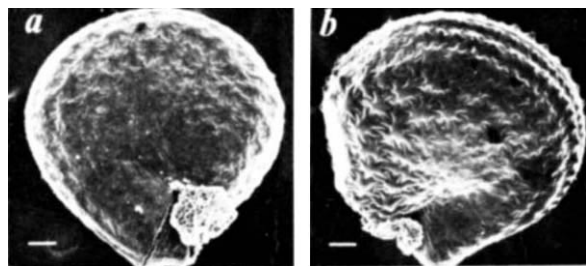


Fig. 3 Scanning electron micrographs of *Portulaca oleracea* seeds. *a*, Fossil seed from Crawford Lake sediment dating 1420-39 AD. *b*, Modern seed from southern Ontario. Scale bars represent 100 µm.

The discovery of purslane pollen and seeds throws new light on what until now have been rather puzzling aspects of the historical record. For example, as early as 1526 Oviedo included purslane in his list of plants native to both Hispaniola and Spain¹². In 1605 Champlain noted that purslane grew among the Indian corn fields near Plymouth, Massachusetts¹³. Similarly, Sagard, who lived with the Huron Indians during the years 1623 and 1624, reported that purslane was a common weed which grew naturally in fields among their corn and pumpkins¹⁴. Sagard's report is especially significant in that the Huron lived only about 150 km north of Crawford Lake, and were probably the direct descendants of the people who had been living at the Crawford Lake site 300 yr earlier¹⁵. As far as the species' presence in Ontario is concerned, it seems reasonable to assume that it came into the area with Indian agriculture.

Unfortunately, the broader question of ultimate origins cannot be answered on the basis of the evidence presented here. What is established is that the species was present on both sides of the Atlantic before 1492. The question is now raised as to how it achieved its wide distribution. One interesting possibility is pre-Columbian transfer by man. If this was the case, however, it must have been by a more southerly route than Asa Gray's Viking hypothesis suggests.

In Europe it does not grow north of latitude 60° and is absent from Iceland, Greenland and Newfoundland¹⁰. Low summer temperatures are probably the limiting factor here as successful germination requires air temperatures greater than 20 °C (ref. 10). A second, perhaps more plausible, explanation is natural dispersal. Purslane seeds are known to be eaten by birds and furthermore have a high viability rate after digestion¹⁰. They are small and presumably could have been carried either internally or externally. Even though purslane's pre-Columbian presence on both sides of the Atlantic may not be attributable to man, the species is still culturally interesting. Like the bottle gourd (*Lagenaria siceraria*), it was independently used in similar ways in both the Old World and the New.

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Suppressive effect of seminal plasma on lymphocyte activation

ALTHOUGH spermatozoa bear histocompatibility antigens (*H-2* in mice^{1,2} and *HL-A* in man^{3,4}) accelerated (immune) skin graft rejection is not known to occur in females previously multiply inseminated by a graft donor. The absence of detectable transplantation immunity in inseminated females correlates with the generally low immunogenicity of spermatozoa given by other routes. The decreased antigenicity of spermatozoa is not explained by a privileged immune status of the female reproductive tract as the vaginal route is adequate for immunisation against a variety of antigens⁵, especially if the tract is wounded⁶, and antibodies to spermatozoa decrease fertility in some situations⁷. Thus, it is probable that mechanisms have evolved to maintain a low immunogenicity of spermatozoa. What seems to be an exception to the low immunogenicity of spermatozoa—that antibody to a tissue-specific sperm antigen occurs naturally without deliberate immunisation^{8,9} and is readily elicited in a variety of species¹⁰—may represent a protective mechanism. Many substances present in seminal plasma may exert a suppressive effect by coating sperm membrane antigens or inducing immunosuppression by other means.

To investigate potential mechanisms for the observed low

Table 1 Human seminal plasma effects on PHA stimulation of peripheral blood lymphocytes

Seminal plasma (%)	No PHA		PHA stimulatory doses				250 µg ml ⁻¹		1,000 µg ml ⁻¹	
	c.p.m.		c.p.m.		c.p.m.		c.p.m.		c.p.m.	
	± s.e.m.	% Inhibition	± s.e.m.	% Inhibition	± s.e.m.	% Inhibition	± s.e.m.	% Inhibition	± s.e.m.	% Inhibition
0	707 ± 93	0	43,565 ± 3,724	0	185,494 ± 13,461	0	117,404 ± 14,611	0		
10%	202 ± 10*	72	2,325 ± 349†	95	71,606 ± 8,696†	62	59,587 ± 13,126*	50		
25%	292 ± 9*	59	15,007 ± 2,540*	66	181,642 ± 5,393	3	138,376 ± 6,317	0		
0.1%	867 ± 388	0	21,142 ± 4,167*	52	176,083 ± 6,188	6	127,674 ± 5,359	0		
0.001%	616 ± 150	13	31,999 ± 6,956	27	171,985 ± 9,325	8	116,210 ± 9,048	0		

Human peripheral blood lymphocytes from normal donors were purified by Ficoll-Hypaque density gradient centrifugation. Lymphocytes were cultured in triplicate in microtitre trays at concentrations of 2×10^5 cells per 0.2 ml RPMI 1640 + 10% pooled human serum. PHA-M (Difco) and serial dilutions of seminal plasma were added at $T=0$ and cultures were pulsed with ³H-thymidine from 66–72 h and collected on glass fibre filters with 0.9% NaCl using a MASH II Automatic Sample Harvester. DNA synthesis was determined by liquid scintillation counting in three separate experiments and is expressed as c.p.m. ± s.e.m.

* $P < 0.02$ against no seminal plasma.

† $P < 0.002$ against no seminal plasma.

immunogenicity of sperm, we have studied the effects of added spermatozoa or seminal plasma on two well defined *in vitro* assays for cellular immunity. First, the mixed leukocyte reaction (MLR) is a model for the afferent limb of natural alloimmunity and specifically detects lymphocyte-defined (LD) transplantation antigens which are genetically determined at the major histocompatibility locus¹⁰. Secondly, phytohaemagglutinin (PHA)-induced stimulation of lymphocyte DNA synthesis is predominately the result of proliferation of thymus-derived (T) cells and is widely employed as a model for antigen-induced lymphocyte transformation. The results demonstrate suppression of the induction of lymphocyte activation in both test systems by seminal plasma, and thereby, provide a partial explanation of the weak immunogenicity of ejaculates of spermatozoa.

Human seminal ejaculates were allowed to liquefy at 37°C for 30 min. Spermatozoa and hyaline bodies were centrifuged leaving a seminal plasma supernatant. The spermatozoa were resuspended in sterile saline, the hyaline bodies allowed to settle out, and the supernatant spermatozoa washed twice in sterile saline, counted in a haemocytometer and adjusted to the appropriate concentration. Mice (Jackson Laboratory, Bar Harbor, Maine) were maintained on normal laboratory feed. Sterile mouse spermatozoa were obtained by dissection of the ducti deferentes and epididymides of 2–8-month-old male mice. Tissue slices (1 mm) were suspended for several minutes in sterile saline, and the tissue fragments removed from the spermatozoa by a No. 16 gauze screen. Sperm were washed, counted in a haemocytometer and adjusted to the appropriate concentration in sterile saline. Dead spermatozoa were prepared by a rapid freeze-thaw cycle and a soluble sperm extract was prepared by taking the supernatant from a 100g, 10 min centrifugation of the frozen-thawed spermatozoa.

Human peripheral blood lymphocytes were isolated from

whole, heparinised blood drawn from normal donors using Ficoll-Hypaque gradient centrifugation¹¹. Murine splenocytes for culture were obtained by teasing the spleen with sterile forceps in Petri dishes. Cells were washed once in RPMI 1640 containing 5% (mouse) or 10% (human) heat-inactivated foetal calf serum, an antibiotic-antimycotic mixture and 100 mM L-glutamine. Responses to either PHA or allogenic cells in the MLR were determined by measuring DNA synthesis in responding lymphocytes.

Preliminary studies were performed to assess the effect of seminal plasma on the response of lymphocytes to PHA. Seminal plasma inhibited basal and PHA-induced DNA synthesis (Table 1), with much greater effects on PHA-containing cultures. The effect was partly dependent on both concentration of PHA and seminal plasma itself. The most marked suppression was detected at a suboptimal PHA dose (25 µg ml⁻¹) but was also noted at optimal (250 µg ml⁻¹) and supraoptimal (1,000 µg ml⁻¹) doses. At higher PHA doses the reduction in inhibition induced by seminal plasma might be explained by competition between the two substances for surface receptors on lymphocytes. We speculate that blockade of DNA synthesis in resting as well as activated lymphocytes may have resulted from prostaglandins present in seminal plasma¹². Prostaglandins have previously been shown to inhibit DNA synthesis in PHA-activated cells by raising intracellular cyclic AMP levels¹³.

We have also studied the effects of seminal plasma on the human MLR. A consistent inhibition by higher concentrations of seminal plasma on the MLR between unrelated donors is shown in Table 2. The single values of separate experiments are given since markedly different stimulation ratios occurred with genetically different donor pairs. Spermatozoa, however, had a variable effect on the human MLR, sometimes even stimulating it, a finding reported by others¹⁴. Since we did not determine the *HL-A* types of the lymphocyte and sperm donors, it was possible that spermatozoal *HL-A* antigens were stimulatory in

Table 2 Inhibition of human MLR by seminal plasma

% Seminal plasma in MLR	Exp.† 1		Exp.† 2		Exp.† 3	
	E/C*	% Inhibition	E/C	% Inhibition	E/C	% Inhibition
Control (no seminal plasma)	37.06 ± 14.89	—	4.22 ± 0.53	—	5.07 ± 0.32	—
0.25%	8.75	77	1.7	60	1.1	78
0.62%	57.00	0	2.9	31	4.3	16
1.25%	6.88	81	<1	100	<1	100
2.5%	6.21	83	1.4	67	1.5	70
6.25%	<1	100	<1	100	<1	100

*E/C = $\frac{\text{experimental}}{\text{control}} = \frac{(\text{c.p.m. human No. 1} \times \text{human No. 2 M})}{(\text{c.p.m. human No. 1} \times \text{human No. 1 M})}$; where M = mitomycin treated

Human lymphocytes (5×10^4) were mixed with 2×10^5 mitomycin-treated lymphocytes in 0.2 ml volume and the indicated amounts of seminal plasma added in 0.05 ml aliquots. The cells were cultured for 120 h, pulse-labelled with ³H thymidine for 6 h, and collected on glass fibre filters with the MASH II. DNA synthesis was determined by liquid scintillation spectrometry.

the MLR. It seemed unlikely that soluble *HL-A* antigens in seminal plasma¹⁵ could explain the inhibitory effects since the seminal plasma antigen would usually be allogeneic and would be expected to have a stimulatory, rather than an inhibitory, effect. On the other hand, it is possible that any potential stimulatory effect on the MLR of soluble *HL-A* antigens was overcome by the inhibitory effect of some factor(s) (such as prostaglandins) in seminal plasma. Alternatively, some of the nucleases¹⁶ or proteolytic activities¹⁷ of seminal plasma may be responsible for the inhibitory effect.

To elucidate the effects of spermatozoa on the MLR in mice, which provide a setting with defined histocompatibility genes, we used CBA/J (*H-2^k*) as the source of responding, and C57BL/6J (*H-2^b*) as the source of stimulating, splenocytes. Preliminary experiments using normal CBA/J splenocytes with mitomycin-treated C57BL/6J splenocytes revealed that DNA synthesis was maximal at 48 h culture and that a 1:1 ratio of stimulating to responding splenocytes gave optimal responses in the MLR. Both live and dead autologous spermatozoa showed statistically insignificant and slight inhibitory effects at low dosages of spermatozoa on the MLR in this *H-2* combination, whereas the soluble sperm supernatant had no effect. Thus, the inhibitory effect of seminal plasma is unlikely to be the result of soluble components of spermatozoa in the seminal plasma.

The inhibitory effect of seminal plasma may be partly responsible for the poor immunological response in males to genital infection with cytomegalovirus¹⁸ or to first infections with *Neisseria gonorrhea*¹⁹. The inhibitory effect of seminal plasma components on the MLR in mice is unlikely to explain the necessity for the large numbers of spermatozoa required to affect decreased fertility in immunised females²⁰⁻²² since epididymal spermatozoa, free of seminal plasma, were used for those experiments. Finally, we conclude that the low immunogenicity of inseminated sperm may be aided by the presence of inhibitory factors present in seminal plasma. A likely candidate is prostaglandin, known to be present in high concentrations¹² and previously shown to exert suppressive effects on lymphocyte activation through cyclic AMP-mediated mechanisms¹³.

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Direct transformation of 3T3 cells by Abelson murine leukaemia virus

MURINE leukaemia viruses (MLV) cause tumours of haemopoietic origin *in vivo*^{1,2}, but although they replicate *in vitro*, they generally do not transform cells. MLV provides helper activity³ for the replication of defective murine sarcoma virus (MSV)⁴ which possess a transforming activity⁵. Abelson and Rabstein⁶ isolated an agent in association with Moloney leukaemia virus (MLV-M) which causes a rapidly progressive lymphoblastic leukaemia of bone-marrow-derived lymphocytes (B cells)⁷. In addition, this Abelson virus (MLV-A) in conjunction with MLV-M causes rapid appearance of immunoglobulin-producing plasmacytomas in BALB/c mice primed with oil⁸. We now report that MLV-A can be defective for virus replication and show that this agent directly transforms 3T3 cells *in vitro*. A quantitative transformation assay is described.

We found that cell-free filtrates of Abelson tumour extracts from BALB/c or Swiss mice consistently transformed NIH/3T3 cells. Transformed foci appeared 3-5 d after inoculation, and were recognised by the presence of rounded and elongated refractile cells separated by cells of normal appearance. Clones of transformed cells (isolated with the use of cloning cylinders) had saturation densities 3-6 times greater than that of untransformed NIH/3T3, demonstrating the loss of density-dependent inhibition of cell division. Also, unlike NIH/3T3, many transformed cells formed colonies in a soft agar gel. Tissue culture fluids overlying cultures of transformed NIH/3T3 cells were used as a source of virus stock for subsequent experiments.

The Abelson leukaemia virus causes the B-cell leukaemia equally well in NIH/Swiss and BALB/c mice⁶, and thus the agent is NB-tropic. To demonstrate the tropism of the transforming activity *in vitro*, we simultaneously infected NIH/3T3 and BALB/c-3T3 cells with serial dilutions of a virus stock. Transforming activity was NB-tropic, for there was only a twofold difference in the frequency of transformation in the two cell lines (Table 1); a tenfold or greater difference would

Table 1 Tropism of the transforming activity

Virus stock dilution*	NIH/3T3		BALB/c-3T3	
	Transformed foci	XC cell syncytia	Transformed foci	XC cell syncytia
1 × 10 ²	79	TMC	31	TMC
3 × 10 ²	17	TMC	8	TMC
1 × 10 ³	5	TMC	3	TMC
3 × 10 ³	1	TMC	0	TMC
1 × 10 ⁴	0	TMC	0	TMC
3 × 10 ⁴	0	34	0	41
1 × 10 ⁵	0	15	0	8
3 × 10 ⁵	0	4	0	3
1 × 10 ⁶	0	1	0	1
Mock infected	0	0	0	0

NIH/3T3 (clone 1)¹² and BALB/c-3T3 (clone A31)¹³ were maintained in Dulbecco's modified Eagle's MEM containing 4.5 g l⁻¹ of glucose and 10% calf serum (Colorado Serum Co.). One day before inoculation, a culture was trypsinised and 10⁵ cells were seeded in replicate 60 mm diameter plastic dishes (Falcon) in medium containing 8 µg ml⁻¹ of Polybrene¹⁴ (Aldrich). The tissue culture medium overlying virus-transformed NIH/3T3 cells was used as a source of virus stock. These stocks were filtered (pore size, 0.45 µm) immediately before use, and serial dilutions of 0.5 ml were used for inoculation. The medium was changed on the fifth day after inoculation. Transformed foci were scored visually on the tenth day on unstained preparations using an inverted microscope at ×100 magnification. The cells were then irradiated with ultraviolet light and overlaid with XC¹⁵ cells. Foci of MLV-M replication were detected as syncytial plaques¹⁵, and scored 3 d later.

*Expressed as the reciprocal of the dilution.

TMC, too many syncytia to count accurately.

Table 2 Inhibition of transformation by anti-MLV-M serum

Virus stock dilution	Anti-MLV-M rat serum		Normal rat serum	
	Transformed foci	XC cell syncytia	Transformed foci	XC cell syncytia
10 ¹	1	TMC	TMC	TMC
10 ²	0	TMC	67	TMC
10 ³	0	22	5	TMC
10 ⁴	0	2	0	TMC
10 ⁵	0	0	0	8
10 ⁶	0	0	0	0
Mock infected	0	0	0	0

Anti-MLV-M serum was prepared in rats bearing tumours caused by the MSV(MLV-M) complex. The serum neutralised MLV-M at a 1:160 dilution but did not neutralise Gross virus past a 1:20 dilution. A 1:40 dilution of anti-MLV-M serum or normal rat serum was incubated with previously filtered virus stock at 25 °C for 30 min. The transforming activity and MLV-M titre were determined in clonal BALB/c-3T3 as described in Table 1.

be expected for a virus with only N or B tropism⁹. The titre of replicating MLV-M in the stock, detected by the XC cell test, was nearly identical on N and B cells and was higher than that of transforming activity. The number of transformed foci was inversely proportional to virus dilution, suggesting

the untransformed culture that gave the positive XC cell test developed the thymic tumours of Moloney leukaemia after 3 months.

Thus MLV-A can replicate in tissue culture and seems to cause morphological transformation *in vitro*. To confirm that MLV-A has transforming activity, we isolated a clone of virus-transformed cells (termed ANN-1) that failed to secrete XC plaque-forming virus. Tissue culture fluids prepared from ANN-1 neither transformed NIH/3T3 cells nor caused Abelson leukaemia when inoculated into mice (Table 4). As no C-type particles were observed by electron microscopy and as tissue culture fluids were free of viral reverse transcriptase, this permanent clonal line is termed a non-producer¹¹. The ANN-1 line is tumorigenic, for 1 × 10⁶ cells readily cause tumours (fibrosarcomas) when inoculated intraperitoneally into newborn Swiss mice. A clone of MLV-M, isolated independently of MLV-A, was used to infect the ANN-1 cells. The infected cells produced an agent that transformed NIH/3T3 and caused Abelson leukaemia (Table 4). Gross virus also rescued MLV-A. An independently isolated non-producer also replicated a transforming virus that caused Abelson leukaemia virus after infection with MLV-M. Thus MLV-A has a transforming activity and can be defective for virus replication. Although the mechanism of leukaemogenesis remains unclear, it is likely that the transforming activity of MLV-A is needed to cause the murine B-cell leukaemia.

Table 3 End point dilution of the transforming and leukaemogenic activities of MLV-A

Virus stock dilution	Titre Transformed foci	XC cell syncytia	Properties of mass culture		Abelson leukaemia No. with leukaemia/ No. of survivors
			Morphological transformation	XC cell test	
1 × 10 ⁴	70	TMC	+	+	6/6
3 × 10 ⁴	28	TMC	+	+	ND
1 × 10 ⁵	17	40	+	+	6/8
3 × 10 ⁵	2	5	+	+	7/10
1 × 10 ⁶	0	2	—	+	0/21*
3 × 10 ⁶	0	0	—	—	0/8

Clonal NIH/3T3 cells were infected with a tissue culture preparation of virus stock as described in Table 1. Plaques of XC syncytia were determined on two plates at each dilution 10 d after the adsorption period. Transformed foci were scored 15 d after the adsorption period on two plates; 15 d later cells from the latter plates, at each virus dilution, were pooled and grown to mass culture. The cultures were examined for the presence of transformed cells, and a subculture of each was overlaid with XC cells to detect replicating MLV-M. Media overlying the mass cultures was filtered (pore size, 0.45 µm) and 0.1 ml was inoculated intraperitoneally into Swiss mice before the second day of life. Three to four weeks later, the animals were killed and autopsied, and the number with leukaemia was compared with the number of survivors. Animals scored as having Abelson leukaemia had gross and histological evidence of lymphoblastic infiltration of the marrow, lymph nodes and meninges, with no involvement of the thymus⁶.

*Sixteen animals developed thymic tumours, which were detected 3 months after inoculation¹.

ND, not determined.

that one agent alters the growth properties of these cells. MSV similarly transforms some permanent cell lines with one-hit kinetics¹⁰.

Anti-MLV-M serum inhibited the transforming activity of virus stock 500-fold and reduced the number of XC plaques 40-fold (Table 2), suggesting that the focus forming agent has one or more coat proteins in common with MLV-M.

To determine whether leukaemogenic activity correlates with transforming activity, we used the end point dilution method. NIH/3T3 cells were infected with serial dilutions of virus stock. Transformed foci were absent when the dilution exceeded 3 × 10⁵, while a few XC plaques were present at 1 × 10⁶ (Table 3). Cells from the plates at each dilution were pooled and grown to mass culture to provide virus for inoculation of animals. The mass cultures at 3 × 10⁵ or less were largely composed of transformed cells; the cells from the 1 × 10⁶ dilution were not transformed, but gave a positive XC test. Only the animals inoculated with the virus prepared from the transformed cells developed Abelson leukaemia, which was noted at 1 month. Although virus prepared from untransformed cultures did not cause this disease, 80% of animals inoculated with virus from

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Table 4 Rescue of MLV-A with MLV-M

Cell line	Addition of MLV-M	Replication of transforming activity	Abelson leukaemia
			No. with leukaemia/ No. of survivors
ANN-1	No	—	0/19
	Yes	—	17/21
NIH/3T3	No	—	0/6
	Yes	—	0/9

The ANN-1 (Abelson-NIH/3T3-Nonproducer) clonal cell line was isolated by infecting NIH/3T3 cells with a 1 × 10⁷ dilution of virus stock; MLV-M could not be detected at a 3 × 10⁶ dilution. The replication of the transforming and leukaemogenic activities were assayed as described in Table 3.

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***In vitro* expression of erythroid differentiation induced by Friend polycythaemia virus**

WITHIN a few days after infection of susceptible mice with the Friend polycythaemia virus (FVP), a large increase in red cell production occurs leading to polycythaemia¹⁻³. The mechanism by which FVP induces this effect is not known although it has been shown that this induction does not result from the presence of erythropoietin (Ep) in the inoculum and is not dependent on the endogenous production of Ep (refs 1 and 3). As yet, no system for both infection *in vivo* and subsequent expression of erythroid differentiation *in vitro* has been described. We have developed a method whereby infection of spleen cells can be accomplished *in vivo* and the late events of erythroid differentiation can be studied *in vitro*. We report that spleen cells cultured soon after infection *in vivo*, before increases in haemoglobin synthesis are detectable, will later increase their rate of haem, or haemoglobin, synthesis *in vitro*. The effect of Ep on the FVP-induced erythroid differentiation was also tested.

Ex-hypoxic plethoric BALB/c mice were used in all experiments. Mice treated in this way have extremely low levels of splenic erythropoiesis⁴. FVP or Ep was administered to the mice at various times before the preparation of spleen cell suspensions for cell culture. The initial rate of ⁵⁹Fe incorporation into haem during the first 4 h of cell culture was used as an indicator of ongoing erythropoiesis. As a measure of expression of the erythroid differentiation *in vitro*, ⁵⁹Fe incorporation into haem was determined after 2 d of cell incubation^{5,6}. Haem radioactivity after the addition of Ep to marrow cells *in vitro* has been shown to be equivalent to haemoglobin radioactivity⁷, and replicate experiments have shown a similar equivalence for mouse spleen cells *in vitro* after FVP infection (W.D.H., and S.B.K., unpublished).

Splenic cell suspensions prepared up to 48 h after infection with FVP (FVP-cells) exhibited a low rate of haem synthesis during the initial 4 h of incubation (Fig. 1*a*). Additional studies have shown that a large increase in the initial rate of haem synthesis is manifest 56 h after FVP infection at the virus dose employed in these experiments. Although the initial rate of haem synthesis by 0-48 h FVP-cells remained quite low, the amount of ⁵⁹Fe incorporation increased markedly when these cells were subsequently incubated for 48 h *in vitro* (Fig. 1*b*).

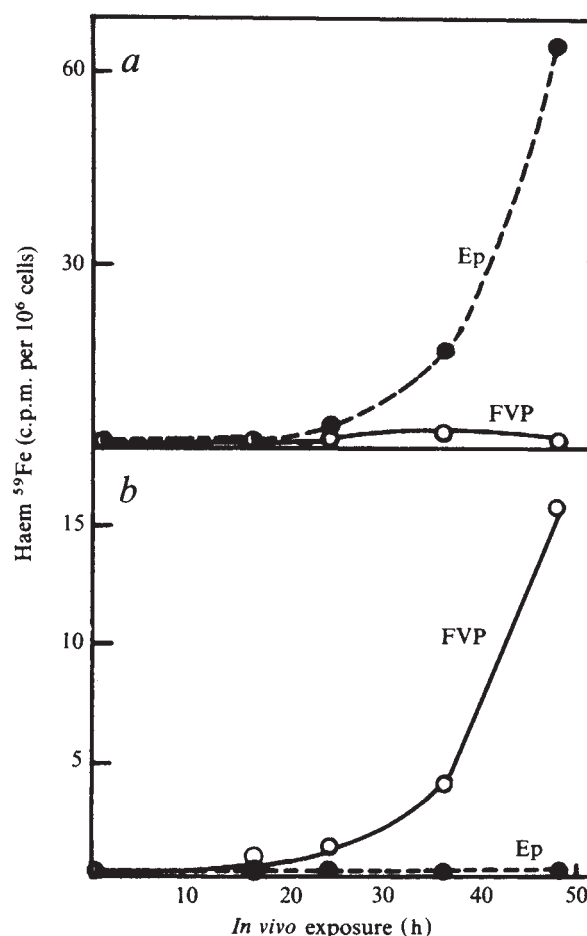


Fig. 1 Effect of FVP or Ep *in vivo* on initial (*a*, 0-4 h ⁵⁹Fe pulse) and late (*b*, 44-48 h ⁵⁹Fe pulse) rate of haem synthesis by spleen cells *in vitro*. All cell suspensions were prepared and cultures initiated simultaneously. At 16, 24, 36 or 48 h before the initiation of the cultures mice were injected intravenously with 50 μ l FVP plasma containing 80,000 focus forming units³ diluted to 0.2 ml with Hanks' solution, or subcutaneously with 3 U of erythropoietin. The zero time controls did not receive FVP or Ep. Spleen cell suspensions were prepared by repeated perfusion of the spleens with Hanks' solution. The cells were collected by centrifugation and washed twice with Hanks' solution. The washed cells were then resuspended in an incubation medium containing 3 U ml⁻¹ heparin, 30 U ml⁻¹ penicillin, and 2 μ g ml⁻¹ streptomycin, and consisting of 60% NCTC-109, 20% human AB plasma and 20% newborn calf serum. Each cell suspension represents the spleen cells from two mice. The cell suspension (1 ml) containing 12×10^6 - 16×10^6 nucleated cells ml⁻¹ was pipetted into Falcon Plastics 35 $\times$ 20 mm tissue culture Petri dishes and incubated at 37 $^{\circ}$ C in an atmosphere of O₂-CO₂ (95%:5%). At 0-4 h (*a*) or 44-48 h, (*b*) 1 μ Ci ⁵⁹Fe, in 0.1 ml medium, consisting of 40% iron-free human AB plasma and 60% NCTC-109, was added to the cells. After 4 h the cells were collected and lysed in dilute Drabkin's solution⁵. Haem was extracted with cyclohexanone and the radioactivity was determined in a gas-flow Geiger-counter with a background of 1-2 c.p.m. as previously described⁶. Each point is the mean of duplicate cultures.

The level of haem synthesis attained in cultures of infected cells increased as the time of exposure to FVP *in vivo* was lengthened. This could be the result of an increased period of infection or an increase in the total time allowing visualisation of a delayed effect. For comparison, cell cultures were also prepared from the spleens of mice treated at varying intervals with Ep (Ep-cells). With these cells, increases in the initial rate of haem synthesis *in vitro* were observed as early as 24 h after the administration of Ep *in vivo* (Fig. 1*a*). The erythropoietic stimulation by the hormone was much more apparent after 36 and 48 h. Other investigators have noted that increases in splenic erythropoiesis are observed earlier after Ep administration than

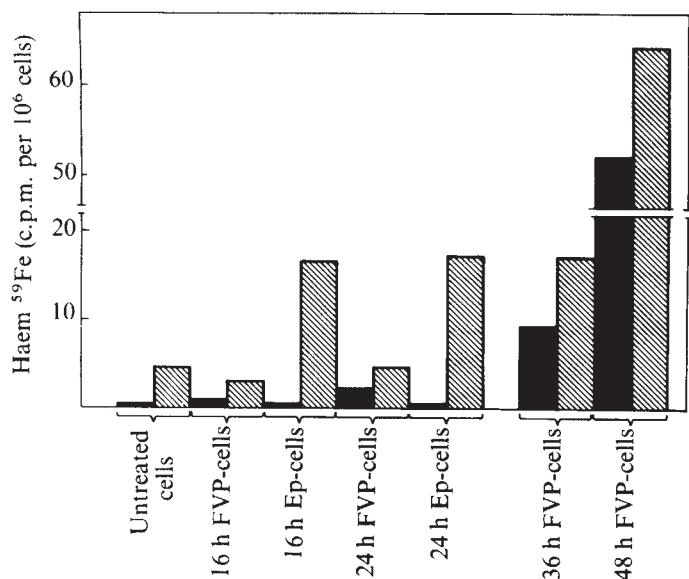


Fig. 2 Effect of FVP and Ep *in vivo* on capacity of cells to respond to Ep *in vitro*. The pretreatment of mice and cell incubation conditions were as described in Fig. 1. Ep (0.48 U ml^{-1}) was added at the time the cultures were started (hatched columns). Controls had no added Ep (solid columns). ^{59}Fe ($1 \mu\text{Ci}$) was added to the plates after 30 h of culture. Sixteen hours later the cells were collected and haem was extracted as in Fig. 1. Each bar is the mean of duplicate cultures.

after administration of FVP (ref. 2). Whether this temporal difference reflects a longer latent period for FVP induction of erythropoiesis or is related to the doses of FVP or Ep used is not known. In contrast to the FVP-cells the rate of synthesis by the Ep-cells after 48 h of cell culture had declined to control levels (Fig. 1b). Experiments are in progress to determine whether the Ep-cells exhibit increased haem synthesis at an earlier point in the culture period.

These experiments indicate that although the rate of haem synthesis has not been altered within 16–24 h after infection of mice with FVP, the spleen cells can increase haem synthesis 48 h later *in vitro*. If virus-induced erythropoiesis is viewed as a process in which cells are infected and induced to undergo erythroid differentiation^{1–3,8} then this system enables the separation of the early events involved in this process from the very late events involved in haemoglobin synthesis. The development of a system in which infection of spleen cells is accomplished *in vivo* and the onset of haemoglobin synthesis is manifested *in vitro* should facilitate the analysis of the biochemical events mediating the virus-induced polycythaemia.

Spleen cells from plethoric mice treated with FVP were also cultured for 46 h in the presence or absence of the hormone, and ^{59}Fe was added to the cells during the last 16 h of cell incubation. In all cases, cultures containing the hormone had higher rates of haem synthesis (Fig. 2). McGarry and Mirand⁹ have previously shown that mouse erythropoiesis *in vivo* was still responsive to Ep early after infection, but the response was lost after several days. Plethoric mice were also treated with Ep 16 and 24 h before placing the spleen cells into cell incubation with and without the hormone (Fig. 2). After 46 h of cell culture without Ep, the cells treated with Ep *in vivo* synthesised only slightly more haem than control cells. Whereas the Ep-cells exhibited a potentiated response to Ep *in vitro* that was much larger than the response of control cells and was similar to the potentiated response observed *in vivo*^{2,10}, the FVP-cells did not show this type of response. Previous treatment with either Ep or FVP *in vivo* produces erythroid differentiation, but these experiments show that a large difference in the two cell populations is manifest when they are incubated with Ep *in vitro*. Ep has been shown to act on the Ep-responsive cells to stimulate their proliferation and increase their number^{11,12}. This could account for the potentiating effect of one dose of Ep on a second, later dose. In this context we conclude that FVP infection does

not produce an increase in similar Ep-responsive cells in the same time as Ep.

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Spectral sensitivity and intensity thresholds in *Nasonia* photoperiodic clock

FEMALES of the parasitic wasp *Nasonia vitripennis* give rise to diapausing progeny (fourth instar larvae) when maintained at short daylength ($< 15.25 \text{ h/24}$) but to continuously-developing or non-diapausing progeny at long daylength ($> 15.25 \text{ h/24}$) (ref. 1). Although the precise anatomical location of this photoperiodic 'clock' has not been determined, it is entirely maternal since the eggs are committed for either diapause or non-diapause development before they are deposited within the host blowfly puparium: by analogy with the aphid *Megoura viciae*² and the silkworm *Antheraea pernyi*³, the clock and photoreceptors are probably in the brain. Photoperiodic time measurement in *N. vitripennis* is known to be a function of the circadian system⁴, and in all probability two separate circadian oscillators, one phase-set by the 'on' transition of the photoperiod and one by the 'off' transition, serve to 'measure' the phase-angle between 'dawn' and 'dusk'⁵. Since, in principle, an experimentally observed action spectrum should correspond directly with the absorption spectrum of the pigment molecule involved and thereby lead to its identification, the spectral sensitivities of the 'dawn' and 'dusk' transitions of the daily photoperiod were determined for *N. vitripennis*.

The ability of insects to discriminate between long and short-day photoperiods using light of different wavelengths has often been studied. Most earlier work, however, was restricted to different wavelengths with no regard to intensity^{6–10}, or to constant light energies at all wavelengths^{11–13}, both of these providing limited information on maximum sensitivities. Indeed, properly conducted energy-compensated action spectra for the photoperiodic response in insects have been determined on very few occasions^{14–17}. Many of these studies have shown an insensitivity to red light^{6–12,14–16}. On the other hand, a number of insects, including the knot grass moth *Acronycta rumicis*⁸, the Colorado potato beetle *Leptinotarsa decemlineata*¹³, the pink boll-worm moth *Pectinophora gossypiella*¹⁸ and the Ichneumonid *Pimpla instigator*¹⁹, are able to respond to wavelengths well into the red end of the spectrum (655–700 nm); and females of *N. vitripennis* have been shown to discriminate between short and long daylengths of red light above 600 nm (ref. 5). Bradshaw¹⁷, working with the reactivation of diapausing larvae of the phantom midge *Chaoborus americanus*, has also shown a maximal sensitivity at longer wavelengths (540 nm)

and a considerable response to red. Evidently, the pigment molecules involved in the insect photoperiodic response absorb maximally in quite different regions of the visible spectrum, and are consequently different in different insect species.

Figure 1 shows that the action spectra for the 'dusk' and 'dawn' transitions in *N. vitripennis* are similar with maximum sensitivity between 554 and 586 nm and considerable sensitivity extending into the red at 617 nm. For the 'dawn' transition (Fig. 1b) the insects responded to about $0.04 \mu\text{W cm}^{-2}$ at 554 nm, 586 nm and 617 nm, and the calculated threshold at 586 nm was in the region of $0.02 \mu\text{W cm}^{-2}$. The threshold at 653 nm was about $0.95 \mu\text{W cm}^{-2}$ but the insects failed to respond to pulses in excess of $30 \mu\text{W cm}^{-2}$ at 765 nm. Females of *N. vitripennis* were also about 20 times more sensitive to a pulse (586 nm) at 'dawn' ($0.02 \mu\text{W cm}^{-2}$) than to one at 'dusk' ($0.46 \mu\text{W cm}^{-2}$).

These action spectra are quite different from those previously described for the photoperiodic reaction. The work of Lees¹⁴ with the aphid *M. viciae*, for example, showed a maximal sensitivity to a 1-h light pulse placed 1.5 h after the end of the 13.5-h main photoperiod in the blue region of the spectrum (450–470 nm). At this wavelength, threshold values (50% virginopara-production) occurred at an intensity of about $0.2 \mu\text{W cm}^{-2}$. Above about 490 nm there was a rapid decline in sensitivity and very little response in the red. Similarly, in the codling moth *Carpocapsa pomonella* and the oak silkworm

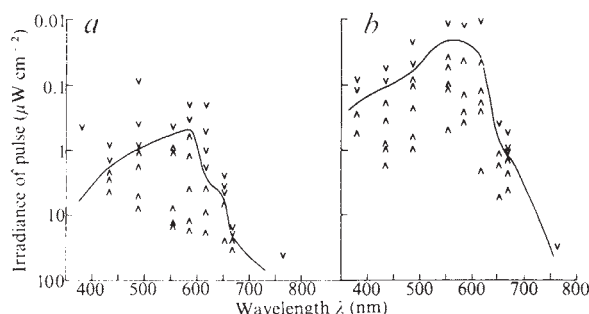


Fig. 1 Action spectra for the 'dusk' (a) and 'dawn' (b) transitions of the daily photoperiod in *N. vitripennis*, monochromatic light of different intensities being offered as a 3-h pulse just after, or just before, a 13 h 'main' light period in each 24 h cycle. v, Groups in which more than 50% of the wasps produced diapausing progeny; Δ, groups in which less than 50% of the wasps produced diapausing progeny. The curve connects those light intensities required to produce a threshold response (50% diapause-producers) in groups of females exposed to wavelengths between 380 and 765 nm. Most groups consisted of 60 female wasps. Groups of *N. vitripennis* were enclosed in glass jars with an ample number of host fly puparia (*Sarcophaga argyrostoma*) in artificial light cycles and at 18 °C for 15 d. At the end of this period, 60 of the surviving female wasps in each group were separated and provided with two fresh hosts for a further 2 d. These puparia, now parasitised, were then removed and incubated at 25 °C in the dark for a further 10 d before being opened to ascertain the diapause or non-diapause status of the progeny. The adult insects were exposed daily to a short-day photoperiod of 13 h white light ($240 \mu\text{W cm}^{-2}$) and 11 h darkness (LD 13:11), supplemented with 3 h of monochromatic light at either the beginning (dawn) or at the end (dusk) of the main photoperiod. The rationale behind this approach was that if the insects saw the supplementary coloured light they would react to the long-day regime (LD 16:8) so produced, and give rise to non-diapause progeny, whereas if they did not see this additional light they would merely respond to LD 13:11 and produce their offspring in diapause. Control groups of LD 13:11 and LD 16:8 (white light) were used. Monochromatic light of different intensities was obtained with the aid of projectors fitted with Osram 6 V 15 W microscope lamps and a series of Balzer narrow-band interference filters coupled with neutral-density filters. The distance between the bulb filament and the experimental insects was about 30 cm. At each wavelength interval the intensity of the light pulse was systematically altered in different experiments until a threshold response (50% of the females producing diapausing progeny) was obtained. The irradiance of each light pulse was measured with a Tektronix digital radiometer in $\mu\text{W cm}^{-2}$.

Antheraea pernyi^{15,16} the action spectra for breaking diapause showed maximum sensitivity between 400 and 500 nm at thresholds between 0.02 and $0.03 \mu\text{W cm}^{-2}$. Therefore, although the intensity thresholds measured here are similar, the pigment in *N. vitripennis* absorbs in quite a different region of the spectrum. In *C. americanus*¹⁷, on the other hand, the maximal response was at 540 nm, closer to that for *N. vitripennis*, but the intensity threshold was considerably lower ($0.001 \mu\text{W cm}^{-2}$).

Since photoperiodic time measurement in *N. vitripennis* involves circadian oscillators^{1,5}, it is likely (as with other circadian systems) that light pulses falling in the early part of the night cause phase delays ($-\Delta\Phi$) whereas pulses falling in the latter part of the night cause phase advances ($+\Delta\Phi$). It is interesting, therefore, to compare the present data with the known action spectra for overt circadian rhythms. The action spectra for the initiation of the rhythm of egg hatch in *P. gossypiella*²⁰ and those for advance and delay phase shifts of the rhythm of pupal eclosion in *Drosophila pseudoobscura*²¹ are all similar, with a peak of sensitivity at 420–480 nm. These action spectra are quite different from those for the photoperiodic response reported here for *N. vitripennis*.

Finally, the low intensity thresholds measured here raise ecological questions concerning the duration of the effective photoperiod and whether moonlight could 'upset' long-night measurement under certain conditions. In *N. vitripennis* the dawn and dusk thresholds occur at 0.02 and $0.46 \mu\text{W cm}^{-2}$, respectively. Bünning²², working in Tübingen (48°N), close to the vernal equinox (March 1–12, 1969), showed that these values occurred approximately 45 min before sunrise (and probably 45 min after sunset). For *N. vitripennis*, therefore, the effective daylength at this time of the year must be regarded as about 13 h 20 min rather than the 11 h 52 min between sunrise and sunset. Similar calculations should be carried out for effective daylengths at other times of the year. As far as moonlight is concerned it is interesting that the dawn threshold is lower than the intensities reached by full moonlight ($0.04\text{--}0.28 \mu\text{W cm}^{-2}$)²³. Interference by moonlight, however, is considered unlikely because photoperiodic thresholds are generally correlated with the ecological habitats and habits of the sensitive stages, those insects in 'cryptic' or shaded situations having a lower threshold than those which are exposed²³. Furthermore, it is known that 8 to 13 long-day cycles are required to reverse the induction of diapause in *N. vitripennis*²⁴ and—because of the period difference between the lunar day (24.8 h) and the solar day (24.0 h)—this number of lunar interruptions is unlikely to occur in the latter half of the night before the period of brightest moonlight encroaches on sunrise and no longer functions as a perturbation.

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Influence of differential chromosome spiralisations on karyotype morphology

THE existence of differential chromosome contraction¹⁻³ presents difficulties for morphometric chromosome analysis. Spiralisations rates of all the chromosomes change simultaneously⁴, and the sizes of all the chromosomes in the set must be correlated with one another, and also with the extent of contraction; the latter may be expressed as the total complement length (the total length of the chromosome complement).

We have measured chromosomes of *Allium cepa* (chromosome complement 52), *Allium fistulosum* (50) and *Macaca mulatta* (28 for female and 23 for male). Since the relationship between chromosome (and arm) length depends on the total complement length, we plotted the absolute length of every identifiable chromosome and its arms, against the total complement length (Fig. 1). Amalgamation of the lines obtained (Fig. 2) shows that there is a linear relationship between the absolute length of each chromosome (or its arm) and the total complement length.

Each linear plot for a chromosome (or arm) has its own angle of slope (α) but this does not seem to depend on the chromosome (arm) length. For any chromosome set, therefore, the mathematical expectation for the size of each chromosome can be determined by measuring any other⁵. This makes it possible to reveal some tiny aberration which cannot be observed by the routine pair or polykaryogram methods, and nomogram chromosome analysis can also be performed.

The correlation between relative chromosome length (ratio

Fig. 1 Correlation between *Macaca* chromosome 1 absolute length and 1 total complement length. 1s, short arm; 1l, long arm; 1, whole chromosome. All graphs were smoothed by a graphic method. The root tips of the onion seeds were pretreated with 0.025% colchicine for 2 h, then with saturated solution of 8-oxyquinoline for 2 h, fixed in acetic acid/alcohol 1:3, stained using the Feulgen method, macerated using cytase and squashed in 45% acetic acid. The cover-slides were separated by the usual dry-ice method and the preparations mounted in Canada balsam. The preparations of the monkey chromosomes were obtained by the Moorhead method with modifications. Chromosomes were measured on the positive prints with magnification $\times 3,400$ (onions) and $\times 3,200$ (monkey) using a glass ruler calibrated in 0.1 mm steps and a stereomicroscope.

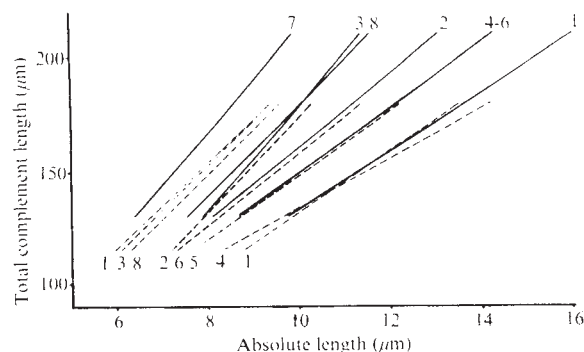
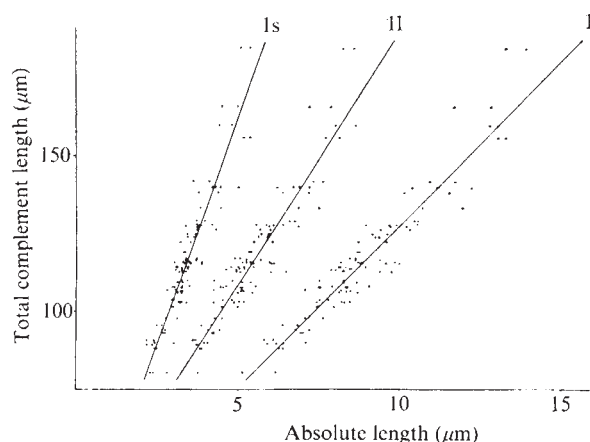


Fig. 2 Dependence of absolute lengths of *A. cepa* (solid line) and *A. fistulosum* (dotted line) chromosomes on total complement length. 1-8, chromosome numbers.

of the chromosome length to the length of complement) and the extent of spiralisations is hyperbolic:

$$l_{rel} = \frac{l_i}{L_i} = \frac{(l_{min} - (L_i - L_{min}) \cot \alpha)}{L_i}$$

where l_{min} is the chromosome length in the most contracted set, L_{min} the total length of this set, l_i and L_i the lengths of the chromosome and the set at any other contraction level. The graphs of change in relative chromosome lengths during spiralisations are segments of hyperbolae (Fig. 3) but there does not seem to be any relationship between chromosome length and spiralisations pattern. The degree of chromosome change is not usually proportional to its length, and sometimes a 'longer' chromosome can become shorter during spiralisations than the 'shorter' one^{5,7}.

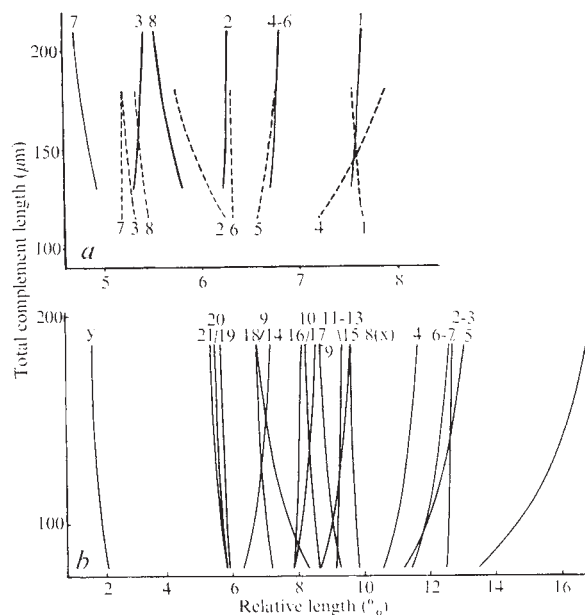


Fig. 3 Dependence of relative lengths of the chromosomes of a, *A. cepa* (solid line), *A. fistulosum* (dotted line); b, *Macaca*, on total complement length.

The correlations between centromeric indices (ratio of the length of the short arm to the whole chromosome length) and the contraction levels are also hyperbolic (Fig. 4). In *Macaca* the differences between the form coefficients of the chromosomes are not so pronounced. This occurs because of an increase in the centromeric indices of subacrocentric chromosomes and a decrease in those of metacentric ones; the latter contradicts the accepted point of view^{2,6}.

Differences in chromosome spiralisation are the result of structural and functional peculiarities of their loci, which can be revealed by the study of the contraction process^{5,7}. The analysis of chromosome parameter dynamics shows that the routine methods of polykaryogram analysis and idiogram construction do not allow the presentation of all karyotype peculiarities because they give only average static patterns from many possible states of the chromosome set.

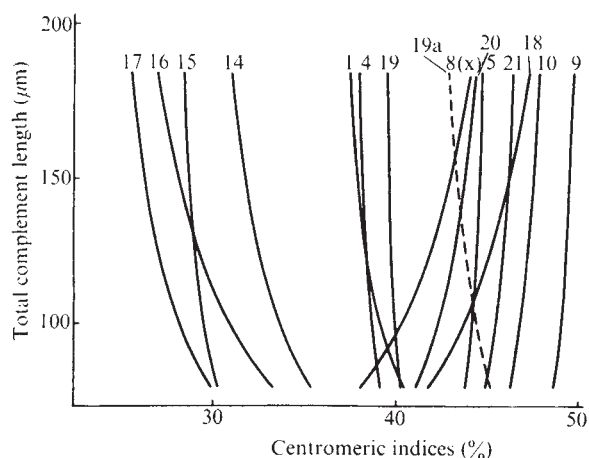


Fig. 4 Dependence of centromeric indices of *Macaca* chromosomes on total complement length.

It also seems that there is sometimes no contraction level which is optimal for the simultaneous morphometric recognition of all the chromosomes. It is possible that some chromosomes which cannot be recognised in less contracted states, differ from one another in the most contracted sets, and vice versa. The idiograms for some chromosomes from the same individual or the same species, constructed for different contraction levels, may differ from one another in relative lengths, form and ability to be identified. From this aspect the optimal representation of karyotype peculiarities is not a static, two-dimensional idiogram, but a three-dimensional idiogram which can show a pattern for every chromosome size and form change during contraction.

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Thymus leukaemia antigen contains β 2-microglobulin

THE major histocompatibility complex (MHC) of the mouse encompasses a number of distinct genetic loci the products of which are expressed on the surface of many types of cell (for review see ref. 1). Some of the gene products may be functionally and structurally related. This is in fact the case for the H-2K and H-2D histocompatibility antigens², which are composed of

two types of polypeptide chain^{3,4}. The larger subunit has a molecular weight of about 50,000 and carries the alloantigenic structures⁵ whereas the smaller subunit has a molecular weight of about 12,000 and seems to be identical to β 2-microglobulin^{3,4}. In view of the involvement of the MHC in several immunological phenomena¹ it is of particular interest that the amino acid sequence of β 2-microglobulin is homologous with those of immunoglobulin G heavy and light chains⁶⁻¹⁰.

It has been postulated that the MHC has arisen by gene duplications¹¹, in which case MHC gene products other than the H-2K and H-2D alloantigens might be expected to share structural features. The thymus leukaemia (TL) antigens in particular seemed likely to repay investigation since these glycoproteins are of a size similar to the H-2 antigens¹²⁻¹⁵. The TL antigens are normally expressed only on thymus cells of some inbred strains of mouse¹⁶. The TL genotype may be universal since leukaemia cells of phenotypically TLA negative mice frequently express the TL antigens¹⁷. We have isolated TL antigens and report that they are composed of two types of subunits, one of which has been identified as β 2-microglobulin.

An antiserum against TL antigens 1 and 3 (ref. 17) was raised by injecting BALB/c mice (H-2^d, TL-2) with A/Sn thymus cells (H-2K^k D^d, TLA-1, 2, 3) at two week intervals for 14 weeks. The antiserum was absorbed with A/Sn spleen and liver cells until its titre tested in a ⁵¹Cr-release cytotoxicity assay¹⁸ on lymph node cells from A/Sn mice was completely abolished. To confirm that all non-TL antibodies had been eliminated, it was demonstrated that no antibodies were bound to C3H thymus cells (H-2^k, TL2) by a sensitive protein A assay¹⁹. The antiserum was, however, highly cytotoxic when examined against A/Sn thymus cells determined both by ⁵¹Cr-release and trypan blue dye exclusion. To obtain highly specific antibodies against β 2-microglobulin a rabbit anti- β 2-microglobulin antiserum was passed over a Sepharose-coupled human β 2-microglobulin column. The eluted antibodies gave a single precipitation line against urinary macromolecules both on immunodiffusion and immunoelectrophoresis³. Cell surface components of A/Sn thymus cells were labelled with ¹²⁵I essentially as described²⁰. Approximately 1×10^6 radioactively-labelled cells were lysed by the addition of NP-40 and the supernatant of the lysate recovered after centrifugation. Aliquots of the supernatant were incubated with TL1,3 antiserum, normal mouse serum (NMS), or alloantisera against H-2K^k and H-2D^d antigens and precipitated by the subsequent addition of rabbit anti-mouse-IgG serum. The precipitates were collected by centrifugation, solubilised, and subjected to SDS-polyacrylamide gel electrophoresis²¹.

A typical result is shown in Fig. 1. The immune complex formed with the TL1,3 antiserum and thymus cell antigens contained two types of molecules of molecular weights of about 50,000 and 12,000, respectively. The same antiserum did not form a complex with spleen cell macromolecules. The spleen cell macromolecules contained H-2 antigens both from the K and D ends, however, since alloantisera against these antigens complexed proteins of expected type (Fig. 1b). This result further corroborates that the anti-TL1,3 serum did not contain detectable antibodies against H-2 antigens.

The large TL antigen polypeptide chain is of a size similar to the H-2 alloantigen-carrying polypeptide chain, in agreement with previous results¹²⁻¹⁵. In addition, the immune complex induced by TL-1,3 antiserum contained a small polypeptide chain of a size similar to β 2-microglobulin. Experiments were therefore designed to investigate the relationship between this polypeptide chain and β 2-microglobulin.

TL antigens were isolated by immunosorbent purification (see legend to Fig. 2). Thymus cell antigens adsorbed to the anti- β 2-microglobulin column contained appreciable amounts of TL antigen since this material could completely abolish the cytotoxic activity of the anti-TLA-1,3 serum. The same material also contained H-2K^k and H-2D^d antigens as determined in a similar way. Spleen cell macromolecules isolated by the same procedure did not impede the cytotoxic activity of the TLA-1,3

antiserum but were very efficient in doing so with relevant H-2 alloantisera.

The immunosorbent-purified thymus cell surface antigens contained a small amount of aggregated material in addition to antigens of three distinct molecular weights, that is 50,000, 37,000, and 12,000, determined by gel chromatography in 6 M guanidine hydrochloride (Fig. 2). The figure also demonstrates that immune complexes are formed with the TL α -1,3 antiserum and contain only the 50,000 and 12,000-dalton components. This result also demonstrates that anti- β 2-microglobulin antibodies will bind to TL antigens. Immune complexes formed with H-2K^k and H-2D^d alloantisera contained, however, all three components (not shown). The 37,000-dalton material is indistinguishable from papain-digested H-2 alloantigens (L.R., L.O., and P.A.P., unpublished).

To further prove that the small TL antigen 1,3 polypeptide chain is identical to β 2-microglobulin this polypeptide chain was isolated from TL α -1,3 ¹²⁵I-labelled immune complexes, and mixed with ¹³¹I-labelled β 2-microglobulin obtained from H-2 alloantigens isolated by immune precipitation. The two small polypeptide chains were subjected to polyacrylamide gel electrophoresis at pH 8.9 in 6 M urea. The ¹²⁵I and ¹³¹I labelled components showed indistinguishable electrophoretic mobility and seemed homogeneous. Another part of the material was reduced, alkylated and digested with trypsin. The peptides were separated by two-dimensional electrophoresis and chromatography, radioactive peptides cut out from the paper and analysed for content of ¹²⁵I and ¹³¹I. All radioactive peptides contained both isotopes in a similar ratio.

The present results demonstrate that H-2 and TL-antigens share β 2-microglobulin as a common subunit. The size of the large polypeptide chain carrying the distinctive antigenic determinants is very similar for the H-2 and TL antigens. This TL polypeptide chain may in fact be as similar to H-2 antigens as are H-2K to H-2D antigens (L.O., L.R., and P.A.P., unpublished). Current work in our laboratory to establish the similarity at the level of primary structure between

Fig. 1 SDS-polyacrylamide gel electrophoresis of immune complexes between anti TL-1,3 (○) or NMS (●) and ¹²⁵I lactoperoxidase-labelled A/Sn thymus cell surface antigens (a); and between anti-H-2K^k (●), anti-H-2D^d (○) or anti-TL-1,3 (□) and ¹²⁵I lactoperoxidase labelled A/Sn spleen cell surface antigens (b). The arrows denote the positions of marker IgG heavy and light chains. Experimental details were essentially as described²².

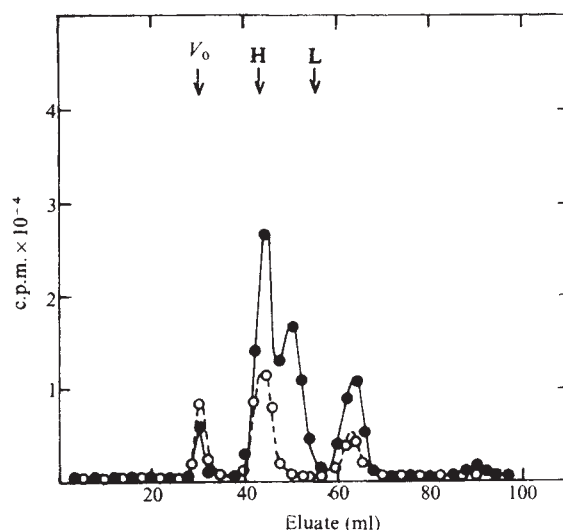
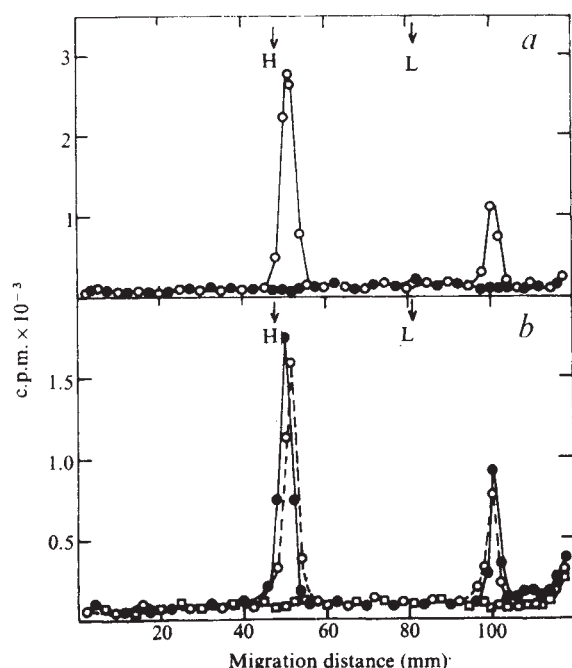


Fig. 2 Gel chromatography of A/Sn thymus membrane antigens on a Sepharose 6 B column equilibrated with 6 M guanidine hydrochloride. Thymus cell membranes were solubilised with 0.5% NP-40 and chromatographed on a column of Sephadex G200. Molecules carrying TL and H-2 antigenic determinants were mainly eluted in a position corresponding to an approximate molecular weight of about 130,000. In the same elution position, the major part of β 2-microglobulin appeared²³. Fractions containing the TL, H-2 and β 2-microglobulin-containing material were pooled, concentrated, passed over a Sepharose-coupled normal rabbit IgG column to remove non-specifically binding material, and subjected to immunosorbent purification on a Sepharose-coupled anti- β 2-microglobulin column²⁴. Material bound to the column was desorbed with 0.05 M sodium citrate buffer, pH 3, containing 0.2 M NaCl. The immunosorbent-isolated proteins were labelled with ¹²⁵I, reduced and alkylated in 6 M guanidine hydrochloride and subjected to gel chromatography in the same solvent (—). Part of the labelled material was complexed with anti-TL-1,3 and precipitated with rabbit anti-mouse IgG. The precipitate was dissolved, reduced and alkylated, and subjected to gel chromatography in 6 M guanidine hydrochloride (---). The arrows denote the elution positions of reduced and alkylated IgG light and heavy chains.

the two types of antigens may lend support to the concept that they have had a related genetic evolution.

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After submission of this manuscript it has come to our attention that Fitetta, Uhr, and Boyse have obtained similar results (*J. Immunol.*, in the press).

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Mechanisms for the excitation of 'free nerve endings'

AMONG the sensory nerve endings of mechanoreceptors there are two principal ways in which excitation can occur. In primary endings like the Pacinian corpuscle, the nerve membrane is directly excited by mechanical deformation conveyed by the surrounding non-nervous cells. In secondary endings like those in the ear, modified non-nervous cells are excited by the stimulus. They then excite the sensory nerve membrane either by current flow from cell to cell, or by extracellular field potentials, or finally by some secretory action which is often by way of a chemical synapse but could be of a more diffuse nature¹⁻³. For fine, unmyelinated sensory nerve fibres, however, the dendrites of which branch in tissues to form 'free nerve endings', it is not clear whether excitation is direct or indirect, or whether the endings are primary or secondary. Here I describe a preparation in which the activity of unmyelinated sensory neurones can be recorded simply and where the question is asked: are 'free nerve endings' excited directly by mechanical stimuli or is their excitation dependent on an electrical depolarisation of the cells that they innervate?

This preparation is possible because of some peculiarities in the skin of young amphibian larvae. In *Xenopus laevis*, intracellular recording and stimulation has shown that current can pass from cell to cell in the skin and that the inward-facing plasma membrane of the skin cells is electrically excitable and can propagate an impulse which is Na⁺-dependent, overshoots zero potential and has a duration of about 100 ms (ref 4). This impulse can be evoked by electrical or firm mechanical stimulation of the outside skin surface and, once evoked, propagates in all directions through the skin. The surface of the head is innervated by trigeminal ganglion cells in the brain. The unmyelinated axons (diameters about 1 µm or less) of these cells lose their sheath cells and then branch into the skin, where the slightly vesiculated dendrites run between the cells (Fig. 1A). In the cement gland, the axons do not seem to branch but form an enlarged sensory ending (Fig. 1B and my unpublished work). The impulse activity of these sensory endings can be recorded in the isolated head using suction electrodes placed on the cell bodies in the trigeminal ganglion (Fig. 1C and my unpublished work). Some 67 units have been characterised.

Using this preparation, the receptive field and adequate mechanical stimulation for a particular unit innervating the skin can be defined. The innervated cells can then be induced to fire a skin impulse by stimulation inside or outside the receptive field. Such an overshooting impulse, propagating through the receptive field of the unit, will depolarise the skin cells beyond zero and therefore presumably more than would result from local mechanical stimulation of the cells, which does not evoke an impulse. Consequently, if the 'free nerve endings' are excited by any indirect method dependent on depolarisation of the skin cells one would expect that the skin impulse would excite them to fire impulses. Figure 1C shows that the skin impulse causes a large potential change at the recording electrode but no impulses are evoked in the sensory neurones. This applies to neurones innervating the head skin and cement gland. This result was confirmed by the fact that skin impulses evoked by electrical stimulation did not lead to muscular responses in isolated head preparations whereas stroking with a

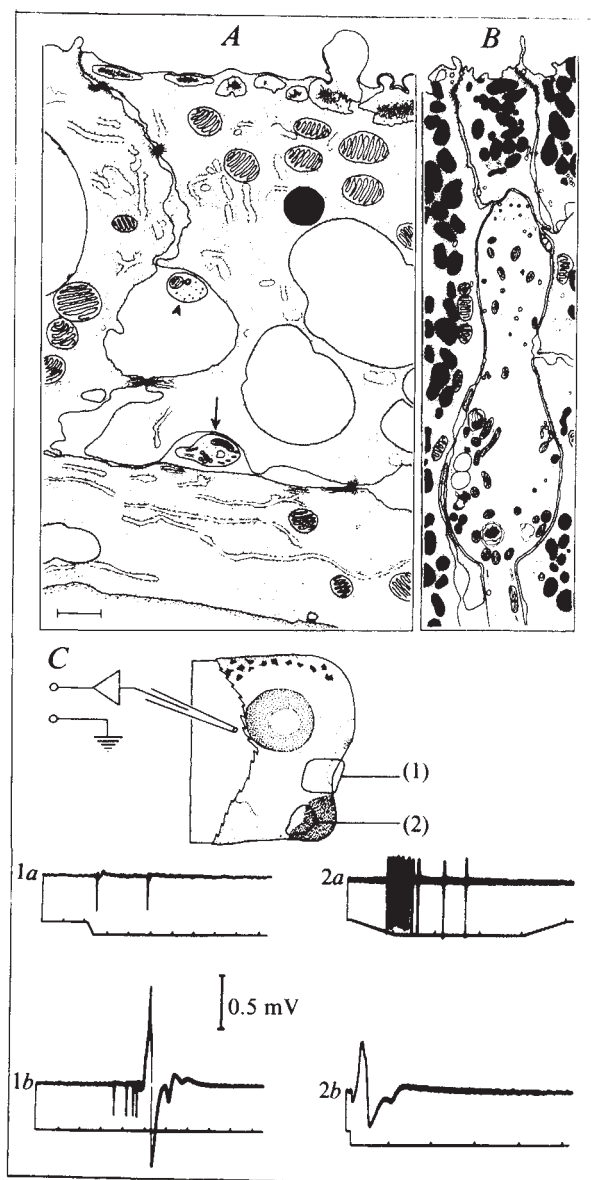


Fig. 1 A and B, Diagrams from electron micrographs to illustrate the two main types of neuronal process found in the skin (A) and cement gland (B). The skin surface is at the top. The sensory dendrites have no sheathing cells but form unencapsulated 'free nerve endings' often closely applied to the cells that they innervate but with no signs of specialised close membrane junctions. The endings contain microtubules and microfilaments (very dense in cement gland endings), clear and dense cored vesicles (20 to 100 nm in diameter), narrow elongated mitochondria, occasional vacuoles and dense bodies (probably lysosomes). In the skin (A) larger sensory processes (arrow) can be seen under the outer layer of cells (with mucus vesicles at their outer surface, black pigment granules and large stippled yolk platelets) while smaller processes (arrowhead) occur between the outer cells. In the cement gland (B) sensory axons form bulbous endings near the secretory surface after running between the elongated gland cells containing numerous black mucus vesicles. There is little extracellular space between mucus cells and the 'free nerve endings'. The 1 µm scale line is at the inner skin surface. C, Diagram to show the isolated head preparation of a young *Xenopus* tadpole. After peeling back the skin behind the eye, sensory activity is recorded in frog Ringer with a glass suction electrode (tip opening 20 µm) on the trigeminal ganglion. The receptive fields of two such neurones are shown with their responses beneath. 1, A unit innervating the skin and responding to a poke in its receptive field with two impulses (1a). A firm stroke evoked four impulses and a skin impulse but no unit impulses during or after the skin impulse (1b). 2, A unit responding to pokes on the cement gland (2a) but giving no response during a skin impulse evoked by a poke to the eye (2b). In each trace the upper beam is the suction electrode record and the lower beam indicates movements of the hair used to deliver pokes plus time marks every 100 ms.

hair to excite the sensory nerves did. This is not the result expected if the skin impulse excites the sensory nerve terminals.

These results indicate that the sensory nerves innervating the head surface of young *Xenopus* (stages 33 to 39) tadpoles⁵ with 'free nerve endings' are not excited by large depolarisations of the skin cells that they innervate. It is therefore very unlikely that the sensory nerve endings are in electrical continuity with the skin cells or that they are excited by secretions liberated from skin cells when they are depolarised by stimulation. It is most likely that the 'free nerve endings' are excited by the direct action of mechanical stimuli in deforming the plasma membrane of the sensory endings. This conclusion could be invalid if skin cells next to sensory nerve processes lost their electrical connections with their neighbours and were not invaded by the skin impulse. In this case many skin cells would be without impulses as the innervation is quite dense (my unpublished work). Though many hundreds of skin cells have been recorded, cells without impulses have not been found in this period of development. Finally, it is possible that mechanical stimulation of the skin could cause secretion without any depolarisation of the skin cells stimulated. If this were the case my conclusions could be wrong.

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Adrenergic receptors in adipose tissue and their relation to adrenergic innervation

THE vascular responses in adipose tissue seem to depend on whether noradrenaline (NA) is released from the sympathetic nerve terminal system or whether it is reaching the receptors by means of the vasculature. Thus electric stimulation of adrenergic nerves to adipose tissue invariably causes α -receptor-mediated vasoconstriction, whereas infusion of NA intravascularly may induce β -receptor-mediated vasodilatation^{1–4}. Likewise intravenous tyramine in monkeys causes vasoconstriction in adipose tissue whereas intravenous NA induces vasodilatation⁵. Since tyramine acts by releasing NA from the sympathetic nerve terminal system, these observations also indicate that NA produces qualitatively different effects depending on how it is delivered to the vascular adrenergic receptors. Our results indicate that the vascular adrenergic α -receptors only are located close to the adrenergic nerve terminal system, whereas the vascular β -receptors may have a different distribution, being farther away from the adrenergic nerve terminals. Consequently, the α -receptors may be affected primarily by NA released from the nerve terminals, whereas the β -receptors are primarily stimulated by circulating catecholamines.

We determined, experimentally, changes in sensitivity to NA in canine subcutaneous adipose tissue following chronic denervation. Blockade of the neuronal uptake by denervation should cause supersensitivity, only at those receptor sites which are in close contact with the adrenergic nerve terminal system^{6,7}. The nerve supplying the inguinal subcutaneous adipose tissue on one side was surgically cut in dogs under Nembutal anaesthesia; the other side was sham operated. Seven to eleven days following denervation the dogs were anaesthetised with Nembutal (20 mg per kg body weight) and the subcutaneous adipose tissues in the inguinal region on both sides were isolated

from skin and underlying fascia⁸. The blood flow was measured by arterial drop recorders. The vein was cannulated with plastic tubing to take blood samples for analysis of glycerol⁹. The nerve was transected on the innervated side. Blood pressure was monitored from a carotid artery and blood pressure and blood flows were recorded continuously on a Grass polygraph. The vasodilatory effect of NA was determined after α -blockade with Hydergin (Sandoz) 120–150 μ g intra-arterially. Lipolytic activity was calculated from the net venous outflow of glycerol. Drugs were administered by close arterial injections.

It was found that chronic denervation caused an increased sensitivity of the adipose tissue to NA as far as lipolysis and vasoconstriction were concerned. The potentiation of lipolysis by denervation was of the same order of magnitude both before and after Hydergin administration. This strongly indicates that blockade of neuronal uptake of NA by Hydergin is of minor importance in adipose tissue (compare ref. 10). Vasodilatation was of the same magnitude in the chronically denervated adipose tissue as in the control tissue (Tables 1 and 2).

Chronic denervation did not change the sensitivity of the tissue to isoprenaline (Tables 1 and 2). This indicates that there was no postjunctional supersensitivity. Thus there was no evidence of superinduction of the lipase following chronic denervation as has been found for *n*-acetyltransferase in the pineal organ of the rat¹¹. Increased lipolysis on administration of NA following chronic surgical denervation may be characterised as a prejunctional supersensitivity, probably due to elimination of the neuronal uptake mechanism. This may also be the explanation for the enhanced vasoconstrictor response. An alternative explanation may be that there is a postjunctional nonspecific supersensitivity of smooth muscles to agonist drugs after denervation. This is not likely, however, since there was no change in the vasoconstrictor response to angiotensin (50–300 ng) or metoxamine (2–6 μ g).

The distance between the nerve terminals and the smooth muscles of the vascular bed is an important factor in determining the role of neuronal uptake in termination of the response^{6,7}. With a large neuromuscular gap the neuronal uptake of the transmitter is of minor importance, whereas the reverse is true when there is a small neuromuscular gap. Our experiments indicate, therefore, that the β -receptors on the adipocytes as well as the vascular α -receptors are located close to the adrenergic nerve terminal system. On the other hand, the vascular β -receptors seem to be unrelated to the adrenergic nerve terminal system.

The finding that intravascular administration of a low concentration of NA induces vasodilatation, indicates that the vascular β -receptors are easily accessible to NA diffusing from the intimal side towards the media rather than from the adventitial side where the adrenergic nerve terminals are located. In

Table 1 Lipolytic responses to intra-arterial injections of isoprenaline (Iso) and noradrenaline (NA) without and during α -receptor-blockade [NA (α -block)] in 12 dogs

Drug	Lipolysis Dose (mole)	Glycerol release (μ mol per 100g)
Iso (n = 6)	4×10^{-11} – 1×10^{-10}	C: 2.6 ± 2.1 D: 1.5 ± 1.1 N.S.
Iso (n = 7)	2×10^{-10} – 1×10^{-9}	C: 28 ± 16 D: 26 ± 17 N.S.
NA (α -block) (n = 6)	4×10^{-10} – 1×10^{-9}	C: 5.1 ± 4.6 D: 11 ± 7.7 $P < 0.02$
NA (α -block) (n = 8)	2×10^{-9} – 1×10^{-8}	C: 18 ± 14 D: 30 ± 16 $P < 0.002$
NA (n = 8)	4×10^{-10} – 1×10^{-9}	C: 7.0 ± 4.2 D: 24 ± 15 $P < 0.01$
NA (n = 6)	2×10^{-9} – 1×10^{-8}	C: 41 ± 16 D: 79 ± 28 $P < 0.01$

Lipolysis is calculated as the net release of glycerol after each injection. The figures given are the mean $\pm$ s.d. C indicates control side, D the denervated side. Statistical hypotheses were tested with Student's *t* test for paired observations.

Table 2 Changes in vascular resistance after intra-arterial injections of isoprenaline and noradrenaline

Drug	Vascular resistance Dose (mol)	% Decrease in PRU
Iso (n = 9)	2×10^{-11} – 1×10^{-10}	C: 39 ± 13 D: 41 ± 15 N.S.
Iso (n = 7)	2×10^{-10} – 1×10^{-9}	C: 69 ± 13 D: 66 ± 10 N.S.
NA (α -block) (n = 6)	2×10^{-10} – 4×10^{-10}	C: 32 ± 17 D: 32 ± 12 N.S.
NA (α -block) (n = 11)	1×10^{-9} – 10^{-8}	C: 64 ± 12 D: 63 ± 9 N.S.
NA (n = 7)	2×10^{-10} – 4×10^{-10}	% Increase in PRU C: 59 ± 33 D: 92 ± 77 N.S.*
NA (n = 9)	1×10^{-9} – 4×10^{-9}	C: 128 ± 78 D: 210 ± 114 $P < 0.002$

The vascular resistance was calculated as the blood pressure over blood flow and was expressed in peripheral resistance units (PRU). A decrease in PRU indicates vasodilatation, an increase vasoconstriction. The percentage change is calculated from the maximal responses after each injection.

* The increase in PRU was greater on the denervated side in six out of seven experiments.

the latter case the vascular α -receptors seem to be in a better position to be combined with NA. Thus the vascular β -receptors in adipose tissue seem to be functionally related to circulating rather than to neurally released NA. From the functional point of view, therefore, they may be classified as humoral β -receptors whereas the vascular α -receptors as well as the β -receptors on the adipocytes may be classified as innervated receptors.

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Thyrotrophin-releasing hormone and shaking behaviour in rat

THYROTROPHIN-RELEASING hormone (TRH) produces behavioural effects in experimental animals¹ and may have psychoactive properties in man^{2,5}. Winokur and Utiger⁶ and Brownstein *et al.*⁷ have described the distribution of TRH in rat brain, suggesting that TRH has a modifying role in synaptic functions in addition to its effect on the pituitary. The behavioural effects of endogenous TRH release in the brain are not known; however, Prange *et al.*⁸ noted that the systemic administration of TRH to pentobarbital-anaesthetised rats resulted in lacrimation, paw tremor and a peculiar shaking movement of the head and trunk. These behavioural effects were also obtained in partially anaesthetised rats following intracisternal injection of 10 μ g TRH per animal. The TRH-induced shaking was particularly interesting because we have observed this behaviour as a characteristic sign of morphine abstinence in the anaesthetised rat⁹. Here, we have studied the central sites of TRH-induced shaking to determine if these sites parallel the endogenous distribution of TRH in the rat

brain and also to determine if these sites correspond to brain areas where morphine withdrawal shakes are obtained.

Male Sprague-Dawley rats (180–240 g) were anaesthetised with a 2.5% sodium pentobarbital solution, 50 mg kg⁻¹ intraperitoneally, and 30 min later mounted on a small animal stereotaxic instrument (David Kopf, Tujunga, California). TRH (Calbiochem, La Jolla, California) was dissolved in 0.9% saline and injected intracerebrally using the micro-injection technique described by Hedge *et al.*¹⁰. All injections were made bilaterally at a volume of 0.5 μ l per hemisphere. The anatomical sites of injection were verified by histological examination of brains after injection of 0.5% methylene blue dye into control animals (see stereotaxic coordinates in Table 1). After intracerebral injections, animals were taken from the stereotaxic instrument and placed in a supine position. Shaking, defined as any quick rotational movement of the head, shoulders, or body was counted for 15 min.

Table 1 Regional sensitivity of the rat brain to the effects of TRH, 0.5 μ g per animal intracerebrally, on shaking behaviour (n = 8 for each brain area)

Target area in brain	Stereotaxic coordinates (mm) [†]			No. of shakes (mean $\pm$ s.e.)
	Ant.	Lat.	Vert.	
Saline in PAG4*	-0.5	0.50	5.5	1.2 $\pm$ 0.7
Substantia nigra	1.75	2.25	6.5	4.1 $\pm$ 2.4
Fasciculus retroflexus	3.5	0.75	5.5	1.4 $\pm$ 0.9
Medial hypothalamus	5.0	0.50	7.5	22.4 $\pm$ 6.7 [‡]
Medial thalamus	5.5	0.50	4.5	34.2 $\pm$ 9.6 [‡]
Thalamic reticular nucleus	5.6	2.0	5.5	4.0 $\pm$ 1.7
Medial preoptic area	7.25	0.50	7.0	14.9 $\pm$ 3.9 [‡]
Basal ganglia	9.0	2.3	5.5	0.7 $\pm$ 0.4
Frontal cortex	10.0	3.0	2.0	0

*Data for TRH injections into the periaqueductal-fourth ventricle (PAG4) area are shown in Fig. 1; animals in all other brain areas received TRH.

[†]The coordinates, based on the atlas of König and Klippel²¹, refer to anterior (Ant.) from lambda, lateral (Lat.) to the midline and vertical (Vert.) from the dura. The upper incisor bar was placed at 2.4 mm below the interaural line.

[‡] $P < 0.01$ against saline, Mann-Whitney U test according to Siegel²².

Injection of TRH into sensitive brain areas produced shaking, lacrimation, paw tremor and intense shivering. After a challenge dose of 0.5 μ g TRH per rat, we found that brain regions exhibiting a significant degree of shaking were located in the periaqueductal-fourth ventricular spaces (PAG4), the medial thalamus, the medial hypothalamus and the medial preoptic area; the substantia nigra, areas around the fasciculus retroflexus, reticular nucleus of the thalamus, basal ganglia and the frontal cortex were relatively inactive (Table 1). The dose-effect curve for TRH-induced shaking in the PAG4 area is shown in Fig. 1. Using six or more shakes as a quantitative index of response, the median effective dose of TRH for eliciting shaking in the PAG4 area was calculated, according to the method of Litchfield and Wilcoxon¹¹, as 0.12 (0.06–0.23) μ g per rat.

These results indicate that the neuroanatomical sites of TRH-stimulated shaking parallel the endogenous distribution of TRH in the rat brain^{6,7}. The apparent localisation of TRH-sensitive areas in the medial mesodiencephalon and medulla may provide clues to the endogenous functions of TRH. The medial mesodiencephalon and the medulla are generally considered to be important integration areas for the control of shivering^{12–16}. The behavioural syndrome of intense muscular activity and vasoconstriction obtained with intracerebral TRH injections suggest that heat gain mechanisms were activated by TRH. It would be of interest to determine if TRH acts, in its postulated modifying role in synaptic functions, as a

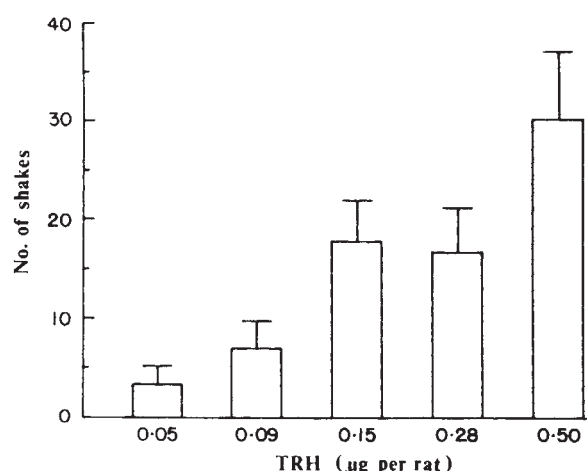


Fig. 1 The dose-effect curve for TRH-induced shaking in the periaqueductal-fourth ventricle area. $n = 8$ for each dose level.

chemical mediator of behavioural and hormonal mechanisms which enable the organism to adapt to cold.

Shaking and vasoconstriction are characteristic abstinence signs which appear in anaesthetised, morphine-dependent rats after the administration of naloxone, an opiate antagonist⁹. The brain areas where naloxone precipitates withdrawal shaking in morphine-dependent organisms¹⁷⁻¹⁹ parallel the sites of TRH-stimulated shaking and the endogenous sites of distribution of TRH (refs 6 and 7). The relationship of some opiate abstinence signs to TRH-linked mechanisms in the brain merits further investigation, since studies by George²⁰ have shown that morphine can act on discrete areas of the hypothalamus to affect the release of thyrotrophin. It should also be noted that the shaking behaviour observed with TRH, and with naloxone in the morphine-dependent animal, exhibits a high degree of chemical specificity, in that various substances such as noradrenaline HCl (0.2 – 5 µg), dopamine HCl (5 µg), L-isoproterenol HCl (5 µg), carbamylcholine chloride (0.2–1 µg), ecothiophate iodide (2 µg), pilocarpine (3 µg), 5-hydroxytryptamine-creatinine sulphate complex (0.02–10 µg), prostaglandin E₁ (5 µg), L-glutamic acid (0.5 µg), L-proline (0.5 µg), L-histidine (0.5 µg), saturated KCl, 2-deoxy-D-glucose (10 µg), 0.2 N NaOH, and 0.2 N HCl, when injected into the PAG4 area under the same experimental conditions as the TRH injections, did not provoke shaking (E.W., and S.S., unpublished).

In summary, we suggest that TRH activates pathways in the brain which lead to heat gain and that the central pharmacological effects of TRH resemble some abstinence signs seen during morphine withdrawal.

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Photopigment conversions expressed in pupil mechanism of blowfly visual sense cells

VISUAL sense cells of flies are depolarised at illumination¹⁻³. This event triggers the pupil mechanism: pigment granules dispersed throughout the cytoplasm migrate towards the rhabdomere, that is, the light-guiding rod-like part of the sense cell which contains the visual pigment molecules⁴⁻⁸. The amount of pigment granules accumulated at the boundary of the rhabdomere controls, through absorption and scattering, the light flux in the photoreceptor⁴.

We have already investigated⁷ the pupil mechanism of the blowfly *Calliphora erythrocephala* by measuring the transmission of the photoreceptors and found that the absorbance of the pigment granules is extreme in the blue and minimal in the yellow. We concluded that, for the photochemical cycle of fly rhodopsin, the visual pigment conversions can be appropriately described by P495 $\rightleftharpoons$ M580; in other words, that

the photoproduct metarhodopsin M580, absorbing maximally in the yellow, can be regenerated into rhodopsin P495 absorbing blue-green, both photochemically ($\rightleftharpoons$) and by a dark process ($\leftarrow$), (refs 7,9,10). We also proposed that, in view of the blue-peaking pupil absorbance spectrum, the essential function of the pupil is a spectral filter action, protecting rhodopsin from excessive photoconversion⁷. Here we present new evidence to support this view, obtained from investigations on the reopening of the pupil during dark adaptation as a function of the previous light adaptation which caused pupil closure.

The pupil was investigated experimentally using the sensitive technique of measuring the antidromic transmission¹¹ of the photoreceptors. The tip of a quartz rod was introduced into the back of the head of the blowfly and a steady test light was applied by way of this rod and the antidromic transmission, that is, the light fraction transmitted by the photoreceptors and leaving the eye through the facet lenses, was detected^{8,7,11,12}. The test wavelength of 508 nm was chosen at about the isosbestic point of the visual pigment⁷, thus avoiding transmission changes resulting from visual pigment conversions; the test light intensity was below the threshold of pupil activation. Activation of the pupil mechanism was induced by irradiating the eye of the fly in the normal, orthodromic¹¹ way for 3 s. Within this light adaptation time the pupil always reached a new equilibrium.

First, intense red illumination, 603 nm, was given to create an almost pure rhodopsin content. After a dark adaptation time t_d , monochromatic illumination of wavelength λ_s closed the pupil, observable as a decrease in transmission. During the subsequent darkness the opening time course of the pupil was determined by measuring the course of the transmission increase. The half time t_k of this opening process (inset of Fig. 1a) is of the order of a few seconds if the preceding illumination

intensity is moderate; but t_k increases monotonically with light intensity, the effect being strongest with blue illumination. The phenomenon of maintained pupil closure notwithstanding renewed darkness we have called the 'glueing effect', because the pupil granules of the visual sense cells seem to remain more or less 'glued' to the rhabdomere.

Figure 1a presents t_k as a function of wavelength λ_s for irradiation of extremely high intensity, showing that the 'glueing effect' is strongest at about 465 nm. These results were obtained with a fixed dark time, $t_d = 2$ min, whereas those in Fig. 1b were obtained with varying t_d and fixed wavelengths $\lambda_s = 584$ nm and 457 nm, respectively. Figure 1b shows that, while the yellow light, at 584 nm, hardly affects the reopening speed of the pupil even after the longest t_d chosen, the same t_d when followed by blue illumination at 457 nm, induces a very strong 'glueing' effect. In other words, especially blue light has a tremendous capacity for slowing down dark adaptation.

How are the photopigment conversions tied up with the migration of the pupil granules? The simple supposition that metarhodopsin production closes the pupil and that the decay of metarhodopsin during the dark reopens it cannot cover the facts: rhodopsin dark regeneration is far too slow for that, the process having a half time of about 30 min⁷. Furthermore, the spectrum of Fig. 1a has a maximum at 465 nm, distinctly to the left of the rhodopsin peak, which is situated at about 495 nm. Starting as we do with pure rhodopsin an intense blue stimulus induces excessive conversion of rhodopsin into metarhodopsin and we have hypothesised that the lack of the opposite conversion has been responsible for the 'glueing' at the cessation

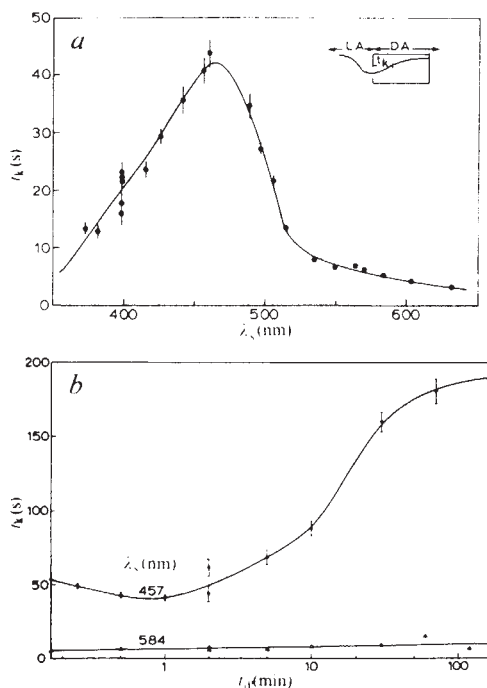


Fig. 1 Reopening of the pupil of the fly after intense illumination. The pigment granules inside the photoreceptor cells of the fly migrate at light adaptation (LA) towards the rhabdomere. The lightflux propagated in and along the rhabdomere decreases as a result of an accumulation of absorbing and scattering pigment granules near the rhabdomere boundary⁴. This transmission decrease (inset) is followed by an increase in transmission during subsequent dark adaptation (DA), as a result of the redistribution of the pigment granules throughout the visual cell soma. The pigment granules thus fulfil a pupil function. The half time t_k of the reopening of the pupil during darkness is determined as a function of the wavelength λ_s of the preceding illumination; this irradiation in turn followed a fixed time of $t_d = 2$ min darkness during which all visual molecules were in the rhodopsin state (a). The same procedure, but now with a varying darkness time t_d and fixed wavelengths, 584 nm and 457 nm, respectively, yielded the t_k values presented in (b). It proves that blue light induces a heavily 'glueing' pupil.

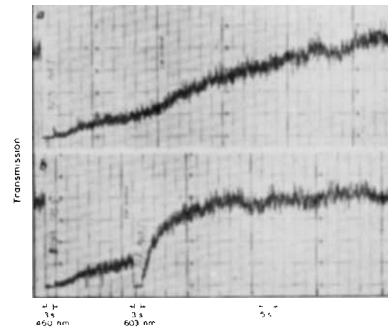


Fig. 2 Experimental transmission curves of the pupil of the blowfly. After a dark adaptation time of 4 min, closure of the open pupil is quickly induced by a 3 s intense blue illumination, 460 nm (during which the photomultiplier is protected from overstimulation). In the following darkness a very slow reopening of the pupil results (a). Repetition of the experiment, but now with an intense red illumination at 603 nm during the dark recovery shows an initial reclosure of the pupil; however, in renewed darkness the pupil shows a markedly enhanced opening speed. Clearly, red irradiation counteracts the 'glueing' effect of the blue light.

of the blue stimulus. Since metarhodopsin is converted into rhodopsin by long wavelength light the hypothesis predicts that red light will reverse the pupil.

To verify this prediction (Fig. 2) we again created pure rhodopsin photochemically, subsequently giving a dark adaptation time $t_d = 4$ min. Having closed the pupil with an intense 3-s blue illumination, $\lambda_s = 460$ nm, another period in the dark results in only a very slow reopening of the pupil (Fig. 2a). An intense red illumination at 603 nm, applied at the onset of this recovery phase promptly closed the partly reopened pupil again, but at the termination of this period the pupil quickly and completely reopened (Fig. 2b). Thus the rhodopsin production caused by the red light might well be responsible for cutting short the 'glueing' effect. We therefore conclude that the two opposite visual pigment conversions induce antagonistic actions in the pupil of the fly.

Antagonistic actions of visual pigment conversions observed in receptor potential measurements in barnacle¹³ and *Limulus*¹⁴ photoreceptor cells must surely be closely related to our results. Antagonistic action of the visual pigment conversions $P \rightleftharpoons M$ and $M \rightleftharpoons P$ on the photoreceptor membrane may occur quite generally¹⁴, although the actual absorption spectra can differ widely: in the barnacle P532 and M495, in *Limulus* ultraviolet-receptor P360 and M480 and in the fly P495 and M580. The two opposite photopigment conversions also influence the early receptor potential in both barnacle¹⁵ and fruitfly¹⁰ photoreceptor cells.

These analogies further support the view that the receptor potential and the force pulling the pigment granules through the cytoplasm of the receptor cell are intimately connected^{5,6,12}. A useful working hypothesis for pigment migration is an electrophoresis model^{5,12} in which it is assumed that on illumination the photopigment conversions locally alter the properties of the receptor cell membrane of the rhabdomere, so that both a changed membrane potential and an intracellular force field are created. In the latter field the presumably charged pigment granules will then migrate towards the rhabdomere. In renewed darkness, recovery of the receptor cell is expressed by a simultaneous dying away of both the receptor potential and the force field, the latter being recognisable as an opening pupil. Brownian motion will disperse the granules until a random distribution is reached.

Encouraging evidence for this model can be drawn from measurements on the receptor potential of the blowfly¹⁶ following the same experimental procedure as that giving rise to Fig. 2a. The time course of the receptor potential was strikingly similar to that of the pupil action.

The benefit derived from the strong bathochromic shift,

occurring at conversion of fly P495 into M580, may now be seen. Both red and yellow screening pigments in the eye of the fly leave the stray red light virtually undisturbed⁷. The meta-rhodopsin, absorbing yellow-red, is therefore converted photochemically into rhodopsin by this stray light, thus favouring dark adaptation (compare Fig. 2).

We have found that dark adaptation is slowed down considerably by blue irradiation. Note that the blowfly pupil absorbance spectrum peaks also in the blue⁷. We therefore conclude that the pupil function is not merely the control of light flux quantity but also the control of its spectral composition in such a way that the cell is not severely desensitised after intense illumination (as for instance by the sun and the sky).

In conclusion, flies seem to have carefully evolved the properties of their photostable pigments in combination with those of their visual pigment. The essential advantage of the pupil to the investigator is that it allows optical probing of visual sense cells and so can reveal further details of both primary visual processes and the generated intracellular force field.

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Opsonin and leukophilic γ globulin in chronically splenectomised rats with and without heterotropic autotransplanted splenic tissue

CHRONICALLY splenectomised animals reflect depressed macrophage activity because the serum immunoglobulins that stimulate them, such as opsonins^{1–6} and leukophilic γ globulins^{7–8}, are depressed. After splenectomy in animals receiving heterotropic autotransplants of splenic tissue, the tissue fragments (after initial necrosis) are reorganised into structures that are microscopically indistinguishable from the original spleen^{9–11}. These reorganised explants also perform erythrophagocytosis and have been known to protect against *Bartonella muris* infections that are usually fatal to splenectomised rats. Furthermore, the cells from these explants can exhibit both humoral and cell-mediated immune reactions¹². I report here that similar explants of autologous splenic tissue can protect against the deficient opsonin and leukophilic γ globulin activity observed in splenectomised rats.

Twenty-four recently weaned Fischer 344 female rats (40–50 g; Charles River Breeding Laboratories) were separated into three groups of eight and one of the following procedures was performed on each animal of a group: (1) sham splenectomy, (2) splenectomy or (3) splenectomy followed by transplantation

of a third of the autologous spleen. Using aseptic techniques, surgical splenectomy (under sodium phenobarbital anaesthesia, 5 mg per 100 g body weight) was performed through a left lateral subcostal skin and peritoneal incision and the spleen was removed. Each rat of one group received approximately one-third of the autologous spleen, which was placed in a subcutaneous pocket made in the left lateral thoraco-axillary region using the blunt end of forceps, and the skin was sutured with autoclips. Three months later preparations of autologous polymorphonuclear (PMN) leukocytes and serum from each animal of each group were used for phagocytosis studies according to the method of Rogers and Melley¹³ as modified by Fidalgo and Najjar⁷.

By intracardiac puncture, blood was obtained from each animal for separation of opsonin and leukophilic γ globulin. Usually about 3 ml of heparinised blood was obtained for cell preparations and 3 ml of clotted blood was used for serum. The buffy coat was removed using siliconised Pasteur pipettes, washed three times (300g) and resuspended using Krebs-Ringer phosphate glucose buffer, pH 7.4 (KRPG). Incubation mixtures consisted of autologous PMN leukocytes and serum (or γ G-globulin) with *Diplococcus pneumoniae* type III (pneumococcus) as target particles. Serum-opsonin activity was evaluated using complement-inactivated (56 °C, 2 h) serum or γ G-globulin (separated by Fahey's method)¹⁴ incubated with PMN leukocytes and unopsonised pneumococcus. Serum leukophilic γ globulin activity was determined using complement-inactivated sera and γ G-globulin preparations (that were 'deopsonised' by exhaustive adsorption with pneumococci). Autologous PMN leukocytes and opsonised pneumococci (incubated with serum at 37 °C for 1 h) were used in these incubation mixtures.

Phagocytosis was studied in silicone-coated glass-stoppered tubes at 37 °C, rotated slowly in a circular rotator. The components of the reaction mixture, added within 1 min, consisted of (1) 0.1 ml leukocytes (2×10^7 – 4×10^7 PMN cells) in KRPG; (2) 0.1 ml of KRPG or 0.1 ml of autologous serum that was either complement-inactivated (for evaluation of opsonins) or in addition, opsonin-depleted (for studies evaluating leuko-

Table 1 Opsonin activity in sera from splenectomised and splenectomised-autotransplanted rats

Rat no.	Cells and KRPG (0.1 ml)	Phagocytosis rates*			
		Complement-inactivated serum (0.1 ml)	Complement-inactivated γ G-globulin (2 mg)		
1	10	22	26		
2	11	24	24		
3	12	20	27		
4 Control	15 Mean	11.0	23 Mean	25.0	25.4
5	14	28	28		
6	10	20	31		
7	12	24	23		
8	14	30	29		
9	10	15	16		
10	11	16	17		
11	13	12	16		
12 Splenectomy	12 Mean	12.8	16 Mean	14.5	15.5
13	12	15	15		
14	15	14	16		
15	14	14	17		
16	15	14	13		
17	10	26	28		
18	12	28	27		
19 Splenectomy-splenic	14	31	30		
20 splenic	15 Mean	13.1	34 Mean	28.0	31.1
21 autotransplant	13	30	31		
22	16	33	34		
23	14	33	33		
24	11	29	34		

Each value represents the mean of triplicate samplings rounded to the nearest number.

*Unopsonised *D. pneumoniae* used in all incubation mixtures.

Table 2 Leukokinin activity in sera from splenectomised and splenectomised-autotransplanted rats

Rat no.	Cells and KRP (0.1 ml)	Phagocytosis rates*	
		Cells and complement-inactivated opsonised serum (0.1 ml)	Cells and complement-inactivated opsonised γ G-globulin (2 mg)
1	16	37	35
2	18	37	38
3	20	38	40
4 Control	21	Mean = 18.5	Mean = 35.1
5	19	31	37
6	17	32	35
7	20	35	34
8	17	35	36
9	16	20	16
10	19	19	21
11	22	20	17
12 Splenectomy	15	Mean = 18.1	Mean = 21.1
13	17	25	23
14	21	21	20
15	17	22	17
16	18	20	21
17	16	40	36
18	22	35	37
19 Splenectomy-	18	39	39
20 splenic	19	Mean = 18.4	Mean = 35.8
21 autotransplant	21	32	34
22	17	34	35
23	16	36	32
24	18	38	35

Each value represents mean of triplicate samplings rounded to the nearest number.

*Opsonised *D. pneumonae* used in cell incubation mixtures.

pilic γ globulin activity), or 0.1 ml of KRP containing 2 mg of autologous γ G-globulin; and (3) 0.1 ml of KRP containing approximately 4×10^7 opsonised bacteria (leukophilic γ globulin activity) or unopsonised pneumococci (opsonin activity). After 30 min of incubation, a sample mixture was removed on a loop of diameter 4 mm and mixed with a similar sample of bovine serum albumin (60 mg ml⁻¹ KRP). The mixture was smeared on coverslips, air dried and stained with Wright's stain. Phagocytosis index was expressed as the number of PMN containing one or more pneumococci per 100 cells counted, 600 PMN cells counted independently each by two observers. All assays were made in triplicate.

Tables 1 and 2 show that sera or γ G-globulin from normal and splenectomised-autotransplanted rats (unlike sera or γ G-globulin from splenectomised rats) had normal opsonin and leukophilic γ globulin activity. The opsonin studies (Table 1) revealed that PMN leukocytes from control animals, in the presence of sera or γ G-globulin, were stimulated into phagocytosis of unopsonised pneumococci from a basal rate of 11.0% (KRP) to 25.0% (serum) and 25.4% (γ G-globulin). Enhanced phagocytosis (from a basal rate of 13.1%) was also observed when autologous serum (28.0%) or γ G-globulin (31.1%) from splenectomised-autotransplanted rats was included in the incubation mixture. Sera or γ G-globulin from chronically splenectomised rats did not enhance phagocytosis (basal rate: 12.8% with (KRP); with serum 14.5%; γ G-globulin: 15.5%). Evaluation of leukophilic γ G-globulin activity from sera of these animals revealed similar findings (Table 2). Autologous PMN leukocytes in the presence of opsonin-depleted sera or γ G-globulin from control animals exhibited a stimulation of phagocytosis of opsonised pneumococci from a basal rate of 18.5% to 35.1% with serum and to 36.9% with γ G-globulin. Rates were similar when serum or γ G-globulin from rats with splenic autotransplants were used (KRP 18.4%; serum 35.8%; γ G-globulin 35.3%). The sera or γ G-globulin from splenectomised rats failed to enhance phagocytosis (KRP 18.1%; serum 21.1%; γ G-

globulin 19.5%). Results were similar when these experiments were repeated.

To evaluate the time necessary for the depletion of serum opsonin and leukophilic γ globulin in rats after splenectomy, 60 recently weaned Fischer 344 females were separated into three groups of 20 and each animal of a group was subjected to either (1) sham-splenectomy, (2) splenectomy or (3) splenectomy followed by autotransplantation of splenic tissue. Opsonin and leukophilic γ globulin activity was evaluated on four rats of each group after 3, 6, 9, 12 and 15 weeks. The sera from sham-splenectomised rats and those receiving autotransplants of splenic tissue exhibited enhanced rates of phagocytosis of pneumococci by PMN leukocytes at all time intervals. The sera from splenectomised animals exhibited optimal opsonin and leukophilic γ globulin activity for 9 weeks followed by a precipitous absence in these activities at 12 weeks and thereafter. When these experiments were repeated the results were similar after 20 and 30 weeks. The serum of these splenectomised animals did not stimulate PMN leukocytosis of pneumococci.

These studies revealed that the depression of opsonin and leukophilic γ globulin activity observed in the sera of chronically splenectomised animals (when compared with the sham-splenectomised controls) could be avoided after ectopic autotransplantation of splenic tissue. The depressed opsonin activity was evidenced by inability of complement-inactivated sera or γ G-globulin to enhance phagocytosis of unopsonised pneumococci by autologous PMN leukocytes. The lack of leukophilic γ globulin activity in sera from splenectomised rats was observed when autologous PMN leukocytes were incubated in the presence of opsonised pneumococci and complement-inactivated sera from which the opsonins had been removed. Optimal opsonin and leukophilic γ globulin activity was exhibited by the sera (or γ G-globulins) from the control and splenectomised autotransplanted rats. The depletion of opsonin and leukophilic γ globulin activity between 9 and 12 weeks following splenectomy tended to parallel the half life of γ G-globulin¹⁵⁻¹⁶. Recent studies performed here suggest that leukophilic γ globulin activity is represented by the γ G₁-globulin moiety of the rat γ G-globulin pool.

The spleen has been known to be essential for the recruitment of lymphocytes to antigenic site for sensitisation^{17,18} as well as production of antibody towards particulate antigens¹⁹⁻²¹. Splenectomised and hereditary asplenic mice reflect defective production of antibodies of the γ -M and γ -G₂ class which may paralleled the lack of thymus-bone marrow synergism that has been observed in these animals²²⁻²⁴. These asplenic mice also exhibit defective production of interferon following infection with Newcastle disease virus that is corrected after neonatal transplantation of splenic cells²⁵. Depressed opsonin and leukophilic γ globulin have also been observed in children who have undergone splenectomy²⁶⁻²⁹. These children exhibit defective phagocytosis and they are also confronted with a high incidence of fulminant and fatal sepsis³⁰⁻³⁴. Whether subcutaneous implantation of splenic tissue (not reflecting hypersplenism) in children undergoing splenectomy would correct these defects remains to be seen.

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Reactivity to tumour-associated antigens detected in mice undergoing liver regeneration

IN addition to the well-known, individually unique tumour-specific antigens in chemically induced neoplasms and the common ones in neoplasms induced by the same virus, there is evidence that neoplastic cells have antigens which are present in certain normal, syngeneic cells, but which may still be reacted against immunologically¹. Most notable has been the demonstration that many tumours share antigens with normal embryonic cells². Immunity to these antigens can be detected in various ways. It has been shown, for example, that lymphocytes from multiparous mice are cytotoxic to cultivated neoplastic but not to normal adult cells³⁻⁵ and that serum from pregnant mice can inhibit (block) this cytotoxic effect⁴⁻⁷.

We describe here data indicating that lymph node cells (LNC) from mice which have undergone partial hepatectomy, followed by liver regeneration, are cytotoxic to cultivated neoplastic cells but not to normal cells and that serum from the hepatectomised mice can block the cytotoxic effect of LNC from multiparous or hepatectomised mice. Our findings suggest that

a normal tissue repair process, involving rapid cell division, may lead to a detectable immunological reaction against antigens present on neoplastic cells.

Partial hepatectomy was performed on BALB/c and C3H mice following established procedures, removing 65-75% of the hepatic parenchyma⁸; the reason for including C3H mice was that these survived hepatectomy better than the BALB/c mice. Littermates of the hepatectomised mice underwent sham-hepatectomy, consisting of anaesthesia, opening of the peritoneal cavity and manipulation of the liver. At various times after hepatectomy, mice were bled from the retro-orbital sinus and their sera stored for later testing of blocking activity. Animals were killed at 7-10-d intervals and tested for LNC-mediated reactivity to cultivated neoplastic cells.

One transplantable BALB/c methylcholanthrene-induced sarcoma line, No. 1315, propagated in culture for approximately one month; a BALB/c Moloney sarcoma virus-transformed 3T3 line (3T3-MSV); BALB/c fibroblasts from the skin of newborn mice, and non-transformed BALB/c 3T3 cells were used as targets in microcytotoxicity assays; this cell material has been described before⁶.

Microcytotoxicity tests for LNC-mediated reactivity and blocking serum activity were performed using Falcon Microtest II plates as previously described⁶; doses of 150,000 and 75,000 (occasionally 25,000) LNC per well and a serum dilution (in tests for blocking activity) of 1:10 were regularly used. The sera were allowed to remain with LNC and target cells for the 30-36-h duration of the experiments.

Absorptions to remove blocking serum activity were performed, as previously described⁶, by incubating 1:5 diluted sera from hepatectomised or control mice for 45 min at 37°C with equal volumes of cell suspensions from regenerating (or normal, control) liver or tumour 1315; the cell suspensions had been prepared mechanically and contained approximately 10⁶ cells per ml. After centrifugation, the absorbed sera were tested for blocking activity.

Table 1 shows that LNC from hepatectomised mice, compared with LNC from sham-operated controls, were cytotoxic to the two tumour lines used as targets. They had no consistent cytotoxic effect against the untransformed, control cells of the same strain as the tumour cells; in some experiments a cytotoxic effect was seen against the control cells which was generally weaker than that detected against the tumour cells, while in other experiments a stimulation of the control cells (negative

Table 1 Cytotoxic effect of lymph node cells from hepatectomised mice on cultivated neoplastic cells

Experiment no.	Days between hepatectomy and test*	No. of effector cells per well × 10 ³	No. 1315 tumour cells	% Reduction of target cells per well	BALB/c fibroblasts	3T3
1	10	150	28.8†	20.6†	15.9	14.0
		75	37.0‡			
	34	150	33.9‡	15.8	7.0	11.5
		75	35.2§			
2	8	150	30.7‡	5.4	33.0‡	0
	18	150	9.1	-26.9	-7.8	-97.3§
	22	150	28.4†	22.6†	25.0†	23.7†
		75	43.2§	40.2‡	-30.5‡	26.7‡
3	10	150	42.5§	13.4	-18.4	1.3
	20	150	28.1†	32.0‡	-9.1	-6.7
	22	150	32.1	38.9§	-3.6	9.2
	81	150	21.8		-10.0	
4	11	150	29.8†		-21.9†	
	13	150	13.4		-21.9†	
		75	-33.5			
	15	150	3.4			
5	17	75	26.7†			
		150	29.1‡		3.3	
		75	46.6§		-14.1	
		25	48.0§		NT	
6	10	150	17.7†		-3.9	
		75				

* C3H mice were used as lymph node cell donors except in part of experiment 1 (34-d mouse), part of experiment 3 (81-d mouse), and experiment 5, when BALB/c mice were used.

Probabilities that differences between experimental groups (hepatectomised) and controls (sham-operated) are due to chance indicated: † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.00$. NT, not tested.

Table 2 Blocking of the cytotoxic effect of lymph node cells from multiparous or hepatectomised mice on tumour cells by serum from hepatectomised mice

Experiment no.	Days between hepatectomy and bleeding*	Blocking effect of 1:10 diluted serum tested with 3T3-MSV			
		% Reduction control serum†	% Blocking by hepatect. serum§	% Reduction control serum†	% blocking by hepatect. serum¶
1	7	1315	10.5	21.7	> 100
	17	19.1‡	52.4		> 100
	21		> 100		> 100
	33		NT		> 100
	66		< --100		> 100
2	7	48.7‡	53.8		
	7-10		67.8		
	21		80.1		
3	80		--7.8		
	11	32.5‡	> 100		
	13		62.2		
4	15		> 100		
	7	17.1‡	--0.6		
	7		27.7		
	19		38.8		
5	23		--86.5		
	8-15	30.8§	43.8		
6	10-15	17.7§	72.3		
7	8-22 absorbed control liver	21.0‡	62.5		
	absorbed regenerating liver		--20.7		
	absorbed 1315 tumour		< --100		
	8-22 absorbed control liver	46.1‡	96.0		
	absorbed regenerating liver		--41.6		
8	absorbed 1315 tumour		14.2		
	unabsorbed		> 100		

* Sera from C3H mice were used except in experiments 6-8 when sera from BALB/c mice were used.

† Serum from matched sham-operated mice used as controls. Percentage reduction of target cell numbers per well with LNC from multiparous or hepatectomised compared with age matched virgin BALB/c females indicated.

‡ LNC from multiparous mice were used for the experimental group.

§ LNC from hepatectomised mice were used for the experimental group.

¶ Blocking serum activity calculated from % reduction with LNC from multiparous or hepatectomised BALB/c in the presence of experimental group (hepatectomised) compared with control (sham-operated) serum. NT, not tested.

cytotoxicity) was observed. Lymph node cell-mediated tumour cell cytotoxicity was seen as early as 8 d and as late as 81 d after hepatectomy. There was no discernable difference whether hepatectomised BALB/c or C3H mice were studied.

Preliminary data indicate that the cytotoxic effect of spleen cells from hepatectomised donors on tumour cells is not removable by passing them through immunoglobulin columns, or by removal of macrophages, while reactivity decreases after incubation of the spleen cells with anti-theta serum and complement. The nature of the effector cells needs to be studied further, however.

Table 2 shows that sera from the hepatectomised mice could abrogate the cytotoxic effect of LNC from multiparous and hepatectomised mice, as compared with sera from sham-operated controls; this was seen whether sera from C3H or BALB/c mice were studied. The blocking effect was detected as a greater number of surviving target cells in the presence of immune lymphocytes and blocking serum than in the presence of immune lymphocytes and control serum; a few of the blocking sera slightly depressed the number of remaining target cells in the presence of control lymphocytes, but most of them did not. Often the blocking serum effect was seen as soon as 7 d after hepatectomy and it remained 2-4 weeks. Two sera taken more than 2 months and one taken 23 d after hepatectomy lacked blocking activity; two of these sera increased the LNC effect.

The blocking activity of sera from hepatectomised mice could be removed by absorption with regenerating liver or No. 1315 tumour cells, while absorption with liver from sham-operated control mice did not remove it.

The specificity demonstrated by the absorption experiments makes it more likely that an immune reaction to some antigens present on tumour cells and regenerating liver cells is responsible for our findings, rather than a more nonspecific destruction of tumour cells, mediated, for example, by activated macrophages. The latter possibility cannot, however, be definitely ruled out and would, if correct, be consistent with the demonstration that

neoplastic cells are more sensitive to a cytotoxic effect of non-specifically activated macrophages than are their non-neoplastic counterparts¹⁰.

One interpretation of the present data, which is worthy of consideration as an impetus for further work, is that undifferentiated cells present in normal, regenerating liver possess antigens which can immunise their host in such a way that both an LNC-mediated cytotoxic response and a blocking serum activity can be measured against neoplastic target cells. One may speculate that this could be because some antigens are shared by tumour cells, embryonic cells and certain rapidly multiplying normal cells. It is interesting in this context that foetal enzymes have been detected in regenerating liver¹¹ and that α -foetoprotein has been demonstrated in the sera of animals undergoing liver regeneration¹². It would then also be tempting to speculate that an immune response to antigens present on certain, perhaps less differentiated, normal cells may provide an "immunostimulation"¹³ of their growth *in vivo*, for example during liver regeneration, and that the blocking serum activity detected *in vitro* may play a role in such a stimulation *in vivo*; there is, however, no direct evidence either for or against this idea. Since a high level of cell multiplication occurs even without partial hepatectomy, for example in bone marrow, intestines and skin, the lymphoid cell-mediated cytotoxicity and blocking serum activity we have detected must be assumed to be the outcome of a change of a normally balanced level, perhaps representing some type of surveillance mechanism.

Finally, we wish to point out that our data suggest one possible explanation why lymphoid cells can be sometimes more cytotoxic to a variety of tumour cells than to normal cells. The cytotoxic effect may have resulted from some repair process, involving stimulation of normal cell growth and immunisation against some antigens present on rapidly dividing cells or, alternatively, the possible activation of macrophages. Our findings do not, of course, argue against the specificity of immune reactions to tissue type specific or individually unique

tumour antigens detected on human and animal neoplastic cells¹.

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Effects of free fatty acid and enzyme release in experimental glucose on myocardial infarction

IN physiological concentrations, circulating long chain free fatty acids (FFA) can inhibit the myocardial oxidation of glucose and are the preferred fuel of the heart in the fasted state^{1,2}. In sufficiently high concentrations, fatty acids as the only fuel can have toxic effects on the normal perfused rat heart³. The mechanisms involved in fatty acid toxicity may include the intracellular accumulation of products of fatty acid metabolism such as acyl CoA (ref. 4), which inhibits the transfer of ATP from sites of synthesis in the mitochondria to sites of utilisation in the cytoplasm. Thus, enhanced rates of FFA metabolism could be expected to aggravate myocardial infarction, in which there is a loss of ATP in the poorly perfused tissue predom-

antly supplied by the occluded artery⁵. As an intracellular ATP deficiency is postulated to be a factor promoting release of enzymes⁶, increased provision of FFA to the heart should accelerate enzyme release from infarcting heart tissue. Conversely, if the rate of accumulation of FFA breakdown products were decreased by the additional provision of glucose or glucose plus insulin to promote intracellular esterification of FFA, then the rate of enzyme release should be decreased. Increased provision of glucose could also result in increased production of anaerobic ATP which could be of importance in promoting the survival of the oxygen-limited myocardium⁷.

We tested these hypotheses in isolated rat hearts, perfused by the left atrium and performing external work⁸, which were subject to ligation of the left coronary artery, thereby reducing coronary blood flow to about 30–46% of its normal flow⁹. Hearts were perfused for 1 h with media containing glucose, or albumin-bound palmitate, or palmitate together with glucose and glucagon-low insulin (Table 1). The rate of release of lactate dehydrogenase¹⁰ was taken as an index of the severity of ischaemic cell damage. Release of lactate dehydrogenase from ligated hearts was four to eight times greater with palmitate-albumin as substrate than with glucose-albumin. Increasing the molar ratio of palmitate-albumin from 1:1 to 5:1 doubled the rate of release of lactate dehydrogenase, in accordance with the effects of higher FFA-albumin molar ratios which accelerate FFA uptake by tissues¹¹. The addition of glucose and insulin to media containing palmitate-albumin decreased the rate of release of lactate dehydrogenase by about two-thirds. Patterns of release of creatine phosphokinase and aspartate aminotransferase were similar to those of lactate dehydrogenase (groups 5 and 6, Table 1); several enzymes are released in parallel in other experimental situations in perfused heart studies¹². Serious arrhythmias, such as ventricular fibrillation, were not found in our experiments although expected at high FFA-albumin molar ratios¹³.

The magnitude of enzyme release from the infarcting heart has been related to the ultimate extent of heart cell necrosis (infarct size) in dogs¹⁴ and in man¹⁵. Our results show that the nature of the substrate reaching the infarcting myocardium can greatly influence the magnitude of enzyme release and can therefore be expected also to influence the ultimate severity and extent of cell necrosis. We provide the first direct evidence for the hypothesis that increased provision of glucose is beneficial and increased provision of FFA is harmful to the infarcting tissue^{16,17} by altering the

Table 1 Effects of various substrates and of insulin on the rate of release of lactate dehydrogenase (LDH) into the medium of isolated perfused working rat hearts with coronary artery ligation

Substrate(s) and insulin concentration	Albumin concentration	FFA-albumin molar ratio†	LDH release U g ⁻¹ h ⁻¹	P‡
1. Glucose 11 mM	0.44 mM*	—	2.6±0.15 (5)	
2. Palmitate 0.5 mM	0.44 mM	1.1	10.7±1.17 (8)	<0.0002 (against 1)
3. Palmitate 1.5 mM	0.44 mM	3.4	19.5±2.51 (4)	<0.004 (against 2)
4. Glucose 11 mM	0.1 mM	—	2.6±0.27 (5)	
5. Palmitate 0.5 mM	0.1 mM	5.0	21.3±1.77 (8)	<0.0002 (against 2)
6. Palmitate 0.5 mM + glucose 11 mM	0.1 mM	5.0	15.3±0.57 (5)	<0.025 (against 5)
7. Palmitate 0.5 mM + insulin 2 mU ml ⁻¹	0.1 mM	5.0	12.9±2.44 (6)	<0.01 (against 5)
8. Palmitate 0.5 mM + glucose 11 mM + insulin 2 mU ml ⁻¹	0.1 mM	5.0	8.1±1.11 (6)	<0.0001 (against 5) <0.004 (against 6)

Enzyme activities measured at 25°C on perfusion fluid sampled from recirculation system. Mean values ± s.e.m. (number of hearts). Substrate concentration = initial value at start of perfusion.

* 3 g 100 ml⁻¹.

† For importance of FFA-albumin molar ratio, see ref. 11.

‡ P values calculated from Student's *t* test for two-tailed values.

Rates of release of creatine phosphokinase were (U g⁻¹ h⁻¹): group 4, 1.2±0.5; group 5, 5.2±1.10 (*P* < 0.01). Rates of release of aspartate aminotransferase were (U g⁻¹ h⁻¹): group 4, 0.8±0.07; group 5, 3.0±0.4 (*P* < 0.001).

extent of metabolic damage. In addition, the decreased rates of enzyme release after the addition of insulin to perfusates containing FFA-albumin in the absence of glucose suggest that insulin has a direct protective action on the ischaemic cell, possibly by inhibition of lysosomal activity¹⁸. Further work is required to determine whether the substrates reaching the heart *in situ* could be changed sufficiently to influence the outcome of myocardial infarction in man.

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Active sodium transport by mammalian urinary bladder

ALTHOUGH sodium reabsorption by the urinary bladder is important to urine formation in amphibia^{1–2}, there have been few studies of ion movements^{3–7} across the mammalian bladder. If the mammalian bladder were an inert sac exhibiting only passive ion fluxes, as generally assumed, ion concentration gradients established between urine and plasma by the kidney would tend to dissipate in the bladder. We now report findings that may resolve this problem: mammalian bladder possesses, in addition to an exceptionally high electrical resistance, an aldosterone-stimulated Na⁺ reabsorptive mechanism.

Sheets of rabbit urinary bladder were mounted *in vitro* between temperature-controlled (37°C) Plexiglass chambers with 2 cm² exposed. The bathing solution was 110 mM NaCl, 7 mM KCl, 25 mM NaHCO₃, 2 mM CaCl₂, 2 mM MgSO₄, 1.2 mM NaH₂PO₄, and 10 mM glucose, gassed with 5% CO₂–95% O₂ (pH 7.4). Three technical problems were encountered. (1) Contraction of the thick muscle layers of intact bladder made membrane characteristics unstable. We therefore dissected away most of the muscle before mounting. The smooth muscle contractant acetylcholine did not affect transepithelial resistance (*R*) or capacitance (*C*) of the resulting epithelial sheet. (2) Ideally, measurements of membrane properties should be normalised to membrane area. The area of epithelium actually exposed in a 2 cm² hole is unknown and variable, because bladder folding varies with stretch. Measurements were therefore normalised to effective epithelial capacitance (*C*, determined from the membrane time constant τ as τ/R)⁸, as a measure of actual epithelial area in cm². For single-cell membranes 1 cm² area corresponds to a capacitance of about 1 μ F (ref. 9). For

bladder, *C* corresponding to 2 cm² chamber area varied with stretch from 1.9 to 5.4 μ F. Such normalised measurements (for example, $\Omega \mu$ F, μ A μ F^{–1}) showed much less variability among preparations than did measurements normalised to chamber area. (3) Edge damage (disruption of cells by the chamber edges, producing high-conductance shunts¹⁰) was avoided by using silicone lubricant (Dow-Corning high vacuum grease) and a flanged chamber design with 20 mounting pins. With these techniques paracellular shunt resistance for rabbit urinary bladder exceeds 300,000 $\Omega \mu$ F, so that edge conductance is undetectable⁸.

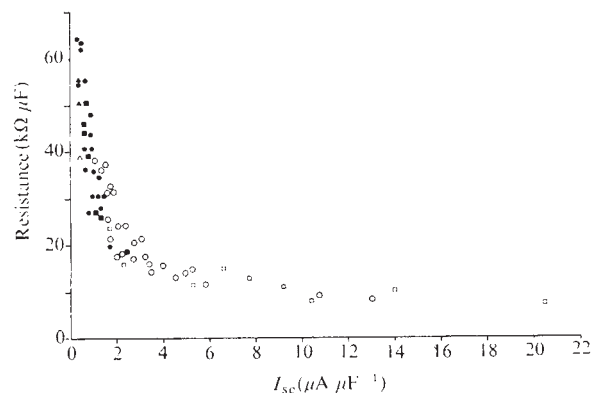


Fig. 1 Relationship between transepithelial resistance (ordinate) and short-circuit current (abscissa) of rabbit urinary bladder, both normalised to effective transepithelial capacitance. Note that *R* and *I*_{sc} are inversely correlated. Animals on normal (□), low-Na⁺ (○), or high-Na⁺ (■) diets; bladder exposed *in vitro* to 10^{–4} M amiloride (●) or 10^{–3} M 2,4,6-triaminopyrimidine (▲) (ref. 15; S. A. L. and J. H. Moreno, unpublished observation); Na⁺ in bathing solutions replaced with choline (△).

Electrical parameters of bladders separating identical solutions varied considerably among animals. The spontaneous potential (*V*) was 24–70 mV (mean $\pm$ s.e.m., 45 ± 2 mV (*n* = 34)), serosal surface (blood side) positive to mucosal surface (urine side). Short-circuit current (*I*_{sc}) was 0.5–20.5 μ A μ F^{–1} (3.0 ± 0.4 (*n* = 34)), the sign corresponding to a net mucosa-to-serosa flux of cations. *R* was 9,000–65,000 $\Omega \mu$ F ($23,000 \pm 1,750$ (*n* = 34)). Spontaneous variations in *I*_{sc} and *R* showed a striking inverse correlation (Fig. 1). *V*, *I*_{sc}, and *R* were approximately constant for 9 h after dissection and then began to decrease.

Three procedures had opposite effects on *I*_{sc} and *R*. (1) Stepwise replacement of Na⁺ with choline in the mucosal solution (but not in the serosal solution) reversibly reduced *I*_{sc} towards zero and increased *R*. (2) When added to the mucosal solution at 10^{–4} M, the natriuretic drug amiloride¹¹ reversibly reduced *I*_{sc} by $85 \pm 9\%$ (*n* = 9) and increased *R* by $93 \pm 45\%$ (*n* = 9), with a half-time of 3 s. (3) At 10^{–6} M the anti-natriuretic hormone aldosterone¹² increased *I*_{sc} by $88 \pm 23\%$ (*n* = 6) and reduced *R* by $35 \pm 6\%$ (*n* = 6), after a lag of 45 min.

These three effects on *I*_{sc} and *R* suggest that the actively transported ion is Na⁺. ²²Na and ³⁶Cl flux measurements confirmed this conclusion. Mucosa-to-serosa flux (*J*_{ms}) of ²²Na varied linearly with *I*_{sc} (Fig. 2), while serosa-to-mucosa flux (*J*_{sm}) of ²²Na was very small and independent of *I*_{sc}. The ratio (*J*_{ms} – *J*_{sm})/*I*_{sc} was 1.03 ± 0.08 (*n* = 28). In contrast, both *J*_{ms} and *J*_{sm} of ³⁶Cl were very small, approximately equal, and independent of *I*_{sc}. (*J*_{ms} – *J*_{sm})/*I*_{sc} for ³⁶Cl was only -0.08 ± 0.09 (*n* = 9). Thus, Na⁺ accounts for all the *I*_{sc}, and Cl[–] accounts for none of it. On open circuit the ratio *J*_{ms}/*J*_{sm} was 7.53 ± 1.45 (*n* = 10), and the net flux (*J*_{ms} – *J*_{sm}) was 1.23 ± 0.14 (*n* = 10) μ A μ F^{–1} for Na⁺ but only 0.15 ± 0.06 (*n* = 9) μ A μ F^{–1} for Cl[–]. Thus the main counter-ion for Na⁺ on open circuit is not Cl[–], but might be either HCO₃[–] or a serosa-to-mucosa flux of K⁺ and/or H⁺.

The inverse relationship between *I*_{sc} and *R* follows the same curve (Fig. 1), whether *I*_{sc} and *R* are manipulated by choline-

for- Na^+ replacement, amiloride, aldosterone or variation among animals. Evidently, rabbits vary individually in a system that includes a Na^+ pump and a Na^+ conductance pathway which are somehow stimulated by aldosterone and inhibited by amiloride. Rabbit urinary bladder epithelium consists of three cell layers in series. Microelectrode techniques⁸ have shown that the open-circuit voltage and transepithelial resistance reside entirely in the cell layer nearest the mucosal solution, and that changes in resistance of the cell membrane nearest the mucosal solution are entirely responsible for the observed variation in R with I_{sc} . Thus, the Na^+ conductance pathway resides in this membrane.

To test for a physiological role of bladder Na^+ transport, we placed rabbits on either a high or low- Na^+ diet for 7–14 d and monitored urine $(\text{K}^+)/(\text{Na}^+)$ ratios as an index of Na^+ retention. At the end of this period the bladder transport properties were studied *in vitro*. The high- Na^+ diet was found to decrease I_{sc} and urine $(\text{K}^+)/(\text{Na}^+)$ (compared with values for animals on a normal diet). The low- Na^+ diet had the opposite effect and probably led to high plasma aldosterone levels, as reflected in the high urine $(\text{K}^+)/(\text{Na}^+)$ ratio¹³. These results suggest that bladder V , I_{sc} , and R respond to physiological release of aldosterone as well as to *in vitro* doses.

Under conditions of restricted Na^+ intake (low urine (Na^+)), a steep concentration gradient exists for passive diffusion of Na^+ from plasma to bladder lumen. The function of the Na^+ pump in bladder is presumably to maintain the urine (Na^+)

perties to membrane capacitance may also be relevant to other epithelia.

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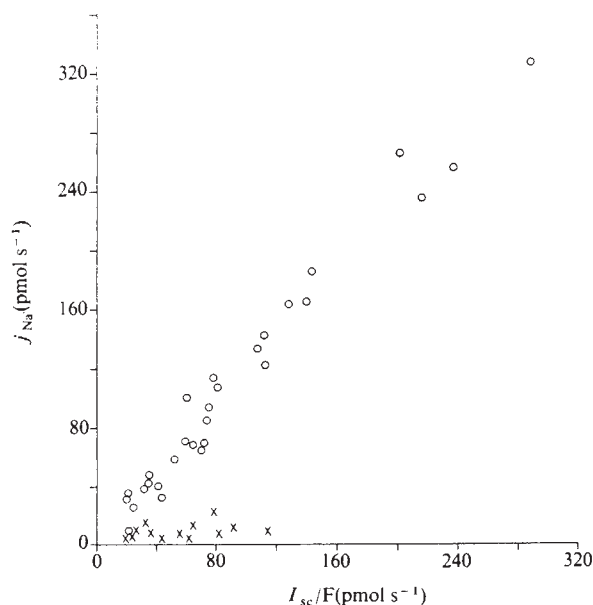


Fig. 2 Relationship between one-way tracer flux of ^{22}Na (ordinate) and total net flux of ions calculated from I_{sc} as I_{sc}/F (abscissa). $\circ$, Mucosa-to-serosa flux J_{ms} ; $\times$, serosa-to-mucosa flux J_{sm} . Note that variation in J_{ms} but not in J_{sm} is closely correlated with variation in I_{sc} .

constant by actively transporting Na^+ from lumen to plasma, thus preventing dissipation of the Na^+ gradient. In addition, most of the ion permeability of the bladder is associated with the aldosterone-stimulated, amiloride-inhibited pathway, through which Na^+ can pass from urine to blood but perhaps not in the reverse direction. When this pathway is inhibited by amiloride or a high Na^+ diet, bladder resistance is very high ($>45,000 \Omega \mu\text{F}$). Thus, the permeability properties of the bladder minimise plasma-to-urine leakage of ions. Because of its high transepithelial resistance, unmeasurably high paracellular resistance, apparent functional equivalence to a single cell layer, and presence of one cell type in this layer, mammalian urinary bladder provides a convenient experimental system for understanding tight epithelia¹⁴. The techniques we developed for minimising edge damage and normalising membrane pro-

Xeroderma pigmentosum variants have decreased repair of ultraviolet-damaged DNA

XERODERMA pigmentosum (XP) is an inherited human disease characterised by the development of pigmentation abnormalities and numerous malignancies on sun-exposed areas¹. Fibroblasts from typical patients with XP are defective in the repair of ultraviolet-produced damage of their DNA: These cells show (with respect to normal fibroblasts) decreased amounts of ultraviolet-induced unscheduled DNA synthesis¹⁻³, less ultraviolet-induced uptake of bromodeoxyuridine into parental DNA (refs 2 and 4), and greater sensitivity to ultraviolet irradiation in terms of colony forming ability⁵⁻⁷. The relationship of the DNA repair defect(s) to the clinical manifestations of XP, however, has been obscured by the finding of a group of patients, designated XP 'variants'⁸, who have the clinical manifestations of the disease¹⁻⁸, but whose cells lack the repair defects (Table 1). Using a sensitive host-cell reactivation technique, I have found that fibroblasts from patients belonging to all five known variant kindreds have defects in the repair of DNA damaged by ultraviolet irradiation.

The host-cell reactivation technique used to estimate repair, presumably DNA repair, has been described previously^{9,10}. Briefly, non-irradiated and ultraviolet-irradiated suspensions of adenovirus 2 (a nuclear-replicating, double-stranded DNA virus) were assayed for their ability to form plaques on monolayers of human fibroblasts. Non-irradiated viruses or viruses repaired by the fibroblasts form plaques on such monolayers. This technique has previously been used to quantitate defective DNA repair in typical XP fibroblasts which had between 3% and 50% of normal host-cell reactivation⁹. Using this technique, I found that fibroblasts from the first reported variant, XP4BE, showed approximately 70% of the repair capacity of normal fibroblast strains⁹. This study was inconclusive as only one variant strain was then available for study. To determine whether the defective repair in this variant was characteristic of all variant strains, four strains from the other four known variant kindreds were obtained: all five variant strains (last column, Table 1) have defective repair manifested by similar degrees of decreased host-cell reactivation of ultraviolet-irradiated adenovirus 2.

Figure 1 shows results from two of the five experiments performed to obtain the data in Table 1. Each frame of Fig. 1 presents survival curves of irradiated adenovirus 2 using two

Table 1 Characteristics of the five known XP variant strains

Fibroblast strain	Ultraviolet-induced unscheduled DNA synthesis	Response to ultraviolet irradiation Ultraviolet-induced incorporation of bromodeoxyuridine into parental DNA	Ultraviolet sensitivity of colony forming ability	Reactivation of adenovirus 2 (% normal)
XP4BE	Normal*	Normal*§	Normal†	64
XP13BE	Normal‡	Normal†	Normal†	60
HG859	ND	Normal§	ND	63
XP7TA	Normal	Normal	ND	57
XP30RO	Normal	Normal	ND	57

*Ref. 1.

†Ref. 8.

‡K. H. Kraemer and J. H. Robbins (unpublished).

§J. D. Regan and R. B. Setlow (unpublished).

||H. Slor (unpublished).

||Ref. 13

ND, not done.

The percentage of normal repair was estimated by dividing the dose at which adenovirus survival was 37% (D_{37}) using XP variant fibroblasts as viral hosts (see legend to Fig. 1) by the average D_{37} obtained using normal fibroblasts ($237 \pm 24 \text{ J m}^{-2}$). XP4BE is from Patient 4, NIH XP Series¹, also designated 1162 (refs 9 and 10). Other fibroblast strains from this patient were designated XP16SF (ref. 8), XW (refs 9 and 10), and patient 4 (ref. 3). XP13BE, from patient 13, NIH XP Series, was obtained from the American Type Culture Collection (CRL 1258). XP14SF (ref. 8) is from the same patient. HG859 is a strain taken from a 25-yr-old girl who has eight siblings, four of whom have XP. This strain and information were kindly given by Dr James German, New York Blood Center, New York. XP7TA was prepared by Dr Hanoch Slor, and was obtained through Dr Colin Arlett, University of Sussex. The family of the patient from whom this strain was started has three of seven children with XP (H. Slor, personal communication). XP30RO was grown out in the laboratory of D. Bootsma from a biopsy taken from a 30-yr-old man, the only one involved in his family (E. A. de-Weerd Kastelein, personal communication) by V. Der Kaloustian, Beirut, Lebanon, and provided by Dr Arlett.

normal and three variant fibroblast strains as viral hosts. The variant strains gave rise to steeper viral inactivation curves than did the normal strains, reflecting less repair of the irradiated virus by the variant than by the normal strains. From such slopes the ultraviolet doses required to reduce the virus survival to 37% (D_{37} s) were calculated. By dividing the D_{37} s obtained with each variant strain by the average D_{37} obtained with the normal strains, the relative host-cell reactivation abilities were obtained (Table 1, last column). Previous measurements using the adenovirus host-cell reactivation test to estimate repair levels in ten normal human fibroblast strains (KD, ND and 1119 included) gave an average $D_{37} \pm \text{s.d.}$ of

$222 \pm 20 \text{ J m}^{-2}$ indicating that the normal strains used here are behaving as expected⁹. The average D_{37} obtained here for the XP variant strain XP4BE of 152 J m^{-2} is in good agreement with the value of $153 \pm 15 \text{ J m}^{-2}$ obtained from analysing 20 previous experiments on this strain.

These host-cell reactivation studies show that all the XP variants are unable to repair ultraviolet-irradiated adenovirus as well as normal cells. They do not indicate, however, which repair process is defective. It has been reported^{11,12} that fibroblasts from three of the XP variants have abnormal post-replication repair, but it is not yet known whether such abnormal postreplication repair is the cause of the decreased host-cell reactivation of ultraviolet-irradiated adenovirus 2 in the variants.

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Poly(A) size class distribution in globin mRNAs as a function of time

STUDIES on the mouse globin mRNAs have shown that specific size classes of poly(A) exist in a population of labelled globin mRNAs (refs 1 and 2). These size classes are present in both α and β globin mRNAs (ref. 1) and the overall size of the poly(A) region in these mRNAs decreases with time¹. As the three discrete size classes previously reported were observed

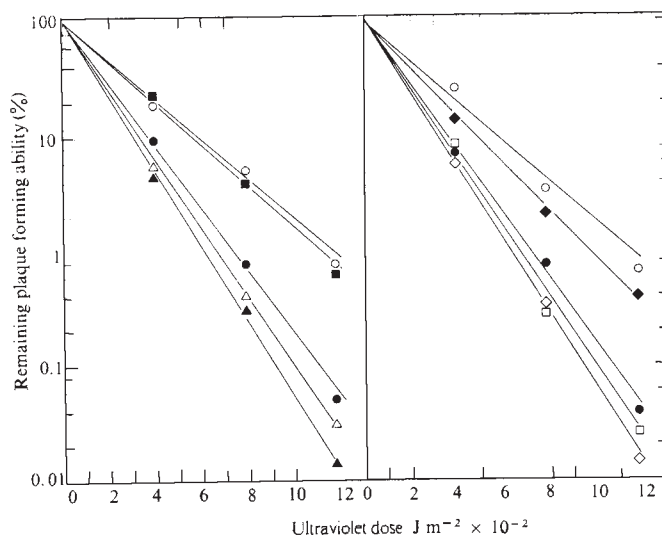


Fig. 1 XP variants' abnormal repair of ultraviolet-irradiated adenovirus 2. Adenovirus 2 was prepared, diluted, irradiated, and plaque on fibroblasts of the various strains as described previously^{9,10}. XP variant strains: ●, XP4BE; ▲, XP7TA; △, XP30RO; ◇, HG859; □, XP13BE. Normal fibroblast strains: ■, 1119; ○, KD; ◆, ND. Average D_{37} is the dose required to reduce virus survival to 37%. Average D_{37} (range), and the number of experiments (n) were as follows. XP4BE: 152 (143-157), $n = 5$; XP7TA: 136 (133-138), $n = 2$; XP30RO: 136 (129-143), $n = 2$; HG859: 149 (137-157), $n = 3$; XP13BE: 143 (139-146), $n = 3$; 1119: 248 (241-254), $n = 2$; KD: 257 (243-275), $n = 3$; ND: 210, $n = 1$; HG800: 212 (207-217), $n = 2$ (data not shown). Normal fibroblast strain HG800 was kindly supplied by Dr James German. The origins of the other normal fibroblast strains have been described^{9,10}.

Table 1 Poly (A) distribution of size classes

Time	Size class	c.p.m.	Specific activity c.p.m. mg ⁻¹	pmol	% pmol	Labelled poly(A) molecules (%)
6 h	Small	602	160	327	79	51
	Intermediate	729	600	53	13	32
	Large	710	600	34	8	17
	Total	2,041	230	414	—	—
10 h	Small	844	435	206	77	62
	Intermediate	819	940	46	16	30
	Large	378	940	14	7	8
	Total	2,031	635	264	—	—
20 h	Small	1,807	1,900	100	78	80
	Intermediate	802	2,100	20	16	15
	Large	451	2,100	8	6	5
	Total	3,060	2,055	128	—	—

Since the amounts of mRNA digested were not equal, the counts from each peak in Fig. 1 were converted to pmol using the specific activity and molecular weight of each size class. Specific activities for small and combined large and intermediate fractions were obtained by Millipore filter fractionation. The percentage labelled poly(A) was calculated from the c.p.m. in each fraction and the molecular weight. This differs from percentage c.p.m. in that the larger size classes will have more counts per molecule.

at one labelling time, we determined whether these same size classes occur at various stages of messenger maturation, as their presence during the overall shortening process could be indicative of a specific degradatory mechanism. The presence of limiting steps in the shortening of the poly(A) region of mouse globin mRNAs is interesting in the light of possible shortening mechanisms and their relationship to poly(A) binding proteins^{3,4} and mRNA stability.

To obtain mRNAs of different age, a ³²P labelling regimen was used which followed the maturation of the mRNA synthesising erythroid precursor cells. When the mRNA was

digested and the poly(A) region analysed the size classes observed previously were present at all times. The amount of the larger size classes, however, decreased at longer labelling times with a corresponding increase in the smallest size class. The change in the distribution of the size classes was calculated to obtain kinetic data for possible models of poly(A) shortening.

Reticulocytosis was induced in Swiss Cox mice as described previously¹. Mouse globin mRNA was labelled *in vivo* by injecting 2 mCi ³²P per mouse at 20, 10 and 6 h before collection of reticulocytes, to insure the same degree of reticulocytosis in all animals at all time points. When an anaemic animal is injected with ³²P, the erythroid precursor cells incorporate the label into RNA; however, only labelled RNA from those cells which can mature between time of labelling and reticulocyte collection will be analysed.

The poly(A) fragments can be categorised into three major size classes based on the 20 h distribution: one migrating slightly heavier than 4S RNA, one migrating intermediate between 4S and 5S and a shoulder migrating heavier than 5S (Fig. 1). These results are consistent with those reported previously for an 18-h labelling period¹. At earlier times the fragments in the shoulder could be tentatively resolved into two or three peaks.

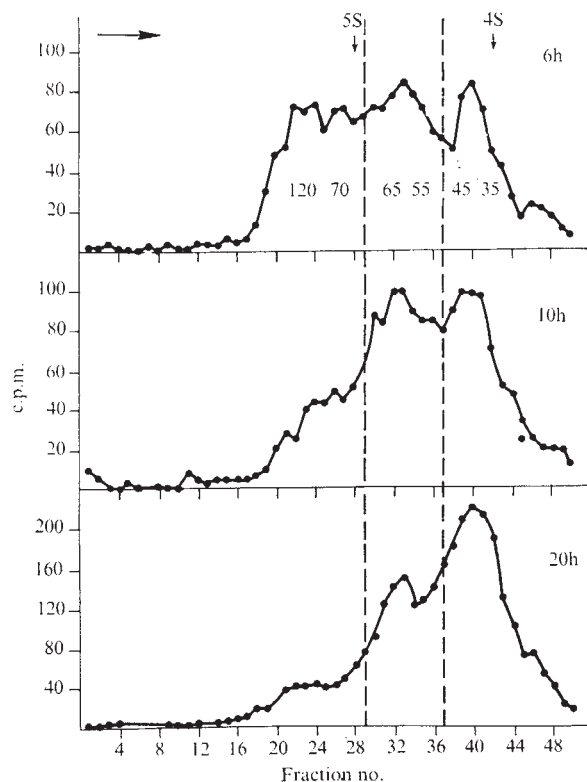


Fig. 1 12% SDS polyacrylamide gels of ³²P poly(A)-containing fragments. Reticulocyte polysomes were collected as described previously⁵. RNA was extracted using the phenol-chloroform-isoamyl alcohol technique⁶ and the globin mRNA isolated by two passages through oligo(dT)-cellulose as described previously⁶. The globin mRNAs were digested with RNase A and T₁ and the poly(A) fragments isolated as described previously^{1,7}. The size of the poly(A) regions was determined using electrophoresis. The fragments are divided into three major size classes (dotted lines) corresponding to lengths of 35–45; 55–65; and 70–120 nucleotides.

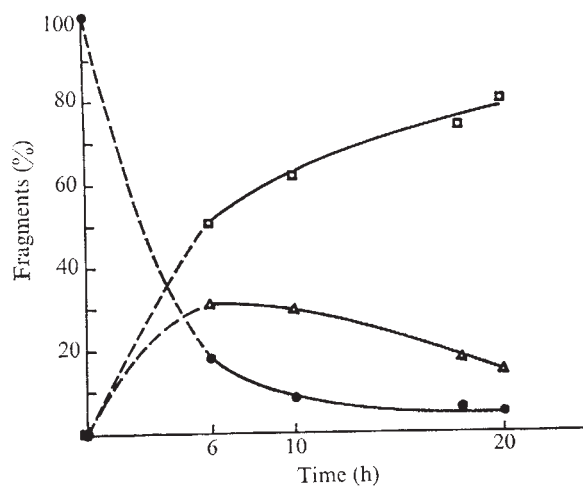


Fig. 2 Plot of percentage poly(A) fragments in each size class present at various times after labelling (last column of Table 1). □, A₃₅₋₄₅; △, A₅₅₋₆₅; ●, A₇₀₋₁₂₀.

For a kinetic analysis of the data it is important to determine the precursor pool. From the specific activity of erythroid precursor cell rRNA and mRNA, it seems that the rate of incorporation is constant for 10 h, indicating that the pool is constant for this time and satisfies the condition for a con-

tinuous labelling experiment. The rate of label incorporated into the reticulocyte RNA is also constant during the times assayed, as the specific activities of reticulocyte mRNA are comparable to those in the precursor cells 6–8 h earlier. This time lag is caused by the maturation of precursors before they reach the circulation. Therefore, the 20 h reticulocyte RNA which was in the spleen 6–8 h earlier is still a product of continuous labelling.

Further data on the poly(A) size distribution was obtained by measuring the specific radioactivity of the short and longer poly(A) size classes (Table 1). The mole fraction of each size class is constant and reflects the steady state distribution of poly(A) sizes in reticulocytes. This is in agreement with the distribution of unlabelled mRNA on Millipore filters. The actual distribution of mRNAs containing each size class is identical at each time point. The only variation is the distribution of radioactivity and this represents the change of labelled poly(A) during the labelling period. This is further evidenced in that at 20 h the distribution of labelled poly(A) finally reaches that of total poly(A), that is, steady state. We therefore plotted the change in distribution of labelled poly(A) molecules (Fig. 2). The data are given as the fraction of labelled poly(A) in each size class with time. The 18-h time point is

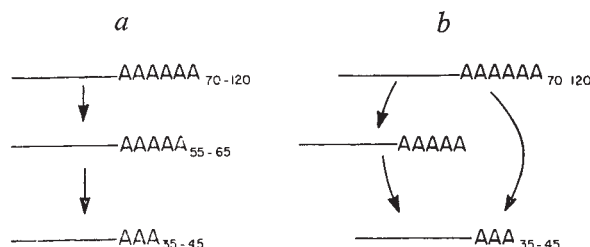


Fig. 3 Two models of poly(A) shortening. *a*, Sequential degradation model would be compatible with the data as a first order process if the curve for the smallest size class showed a sigmoid character before 6 h. *b*, Branched and sequential process would fit the data if the curves before 6 h were as presented.

from our previous data¹, and the 0-h values are based on the assumption that the change in size class distribution is the result of poly(A) shortening and not differential synthesis. This assumption has been validated by recent work using cultured mouse erythroid precursor cells which shows that the poly(A) sequence in newly synthesised globin mRNAs is a single size class approximately 150 nucleotides long⁸.

The decrease in the longer poly(A) size classes in globin mRNAs and the increase in the smallest size class can easily be seen. The data cannot distinguish, however, whether the largest size class gives rise to the intermediate size class alone or to both small and intermediate size classes. The curves before the 6-h time point can only be tentatively assigned because they will differ depending on which of the above two possibilities takes place. *In vivo* labelling experiments cannot provide data before 6 h as this time point is the earliest at which labelled globin mRNA can be isolated from reticulocytes. The observation, however, that at this earliest time point the majority of poly(A) fragments are in the smallest size class, indicates that the shortening takes place in the precursor cells as well as in reticulocytes.

We present two models for poly(A) shortening of globin mRNAs (Fig. 3). In the first model (Fig. 3a) each mRNA molecule is converted to the intermediate with the next longest poly(A) size class, whereas the second (Fig. 3b) allows for some mRNAs to have their poly(A) sequence shortened directly to the smallest size class. Further work on poly(A) shortening in erythroid precursor cells could distinguish between the two models.

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Normal synthesis, transport and decay of mRNA in the absence of its translation

MESSANGER RNA (mRNA) in the cytoplasm of eukaryotic cells constitutes a small fraction of the total RNA synthesised in the nucleus¹. This mRNA seems to be selected from the heterogeneous nuclear RNA (hnRNA) and transported to the cytoplasm where it decays much less rapidly than the hnRNA, which decays in the nucleus. In sea urchin embryos about 10% of the total RNA synthesised in the nucleus becomes cytoplasmic mRNA which decays 10 times less rapidly than the hnRNA. In L cells 2% of the RNA is transported and it decays 50 times less rapidly than hnRNA³. Several proposed models implicate ribosomes or ribosomal subunits in mRNA transport or decay^{4–6}, and we have tested these possibilities in sea urchin embryos using pactamycin to inhibit the association of ribosomes with mRNA⁷ and precursor pool techniques worked out in this laboratory for measuring RNA metabolism². The results show that neither synthesis, transport nor decay of mRNA depend on or are regulated by concurrent protein synthesis.

Pactamycin (4 $\mu\text{g ml}^{-1}$) reduces protein synthesis in sea urchin embryos by more than 95% within 10 min. Polysomes dissociate during the same period. A typical effect of pactamycin is shown in Fig. 1 for polysomes extracted from embryos incubated for 2 h with 10 $\mu\text{Ci ml}^{-1}$ ³H-adenosine. In the cytoplasmic extract of untreated embryos, most radioactive, high molecular weight RNA sediments with the polysomes on sucrose density gradients². In the pactamycin-treated embryos radioactive RNA sedimenting with the polysomes was reduced to the level found in a control extract treated with EDTA to dissociate polysomes. No significant radioactive RNA was associated with the monosomes in the pactamycin-treated embryos. These results establish that pactamycin inhibits protein synthesis and prevents the association of mRNA and ribosomes.

Pactamycin does not prevent the initial binding of mRNA to a ribosomal subunit in some cells⁸. We examined this possibility for sea urchin embryos, since the association of mRNA with even one ribosomal subunit might be sufficient to produce normal regulation of RNA metabolism. Cytoplasmic fractions were prepared from embryos incubated in ³H-adenosine for 30 min, when radioactive RNA is just beginning to appear in the cytoplasm, and for 240 min, when steady state incorporation of radioactivity into mRNA is achieved. These extracts were centrifuged on sucrose gradients for 15 h at 20,000 r.p.m. to sediment the monosomes and subunits to the centre of the gradient. In such conditions even a few per cent of the mRNA would be evident as a peak of radioactivity if it were associated

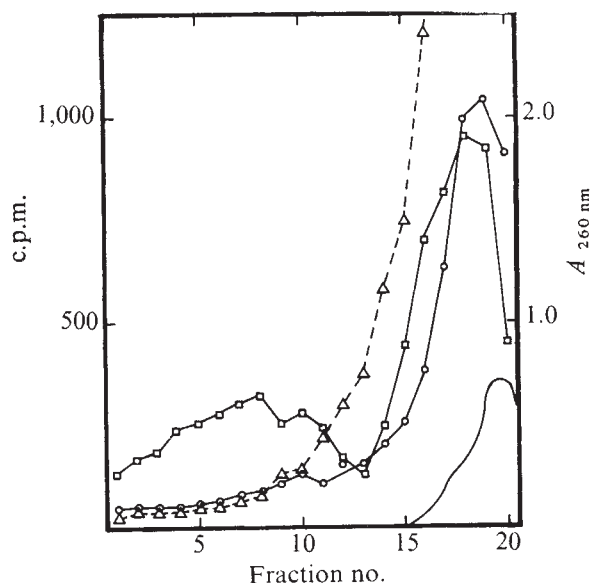


Fig. 1 Sucrose density gradient profiles of polysomes from control and pactamycin-treated sea urchin embryos. Three samples of 5×10^4 mesenchyme blastulae were labelled for 2 h with $10 \mu\text{Ci ml}^{-1}$ ^3H -adenosine. $4 \mu\text{g ml}^{-1}$ pactamycin was added to one sample concurrently with the isotope. The embryos were separated into nuclear and cytoplasmic fractions as before². Briefly, embryos were washed through dextrose several times to dissociate them into cells. They were lysed gently in detergent solution with a Pasteur pipette, layered over a sucrose cushion and centrifuged. This produces a pellet, which constitutes the nuclear fraction, and a supernatant above sucrose, which constitutes the cytoplasmic fraction. There is less than 1% of the total 28S and 18S RNA in the nuclear fraction, and less than 3% of the ^{14}C -thymidine-labelled DNA in the cytoplasmic fraction. All the radioactivity could be recovered in these two fractions. The cytoplasmic fractions were brought to 0.5% Nonidet P-40, one untreated sample was made 0.21 M in EDTA, and each was layered on a 36-ml preformed gradient of 15–35% sucrose in HSB buffer (0.43 M NaCl, 10 mM MgCl₂, 10 mM Tris-HCl, pH 7.4). Samples were centrifuged for 4 h at 24,000 r.p.m. at 4°C. Fractions were collected, and the acid-precipitable counts in each fraction were determined. □, Untreated control; △, EDTA treated control; ○, pactamycin-treated.

with ribosomal subunits (Fig. 2). There was no evidence of radioactive RNA associated with ribosomal subunits immediately after its synthesis or after longer periods in the cytoplasm. Hence, pactamycin prevents any significant attachment of ribosomal subunits to mRNA.

Pactamycin had no effect on the rates of synthesis and decay of total RNA. Sea urchin blastulae, 18–22 h after fertilisation, were incubated with $10 \mu\text{Ci ml}^{-1}$ ^3H -adenosine and samples were removed for RNA and precursor pool measurements. The amount of radioactive RNA that had accumulated after various times of incorporation was calculated from the radioactivity in the RNA and the specific activities of the ATP pools (Fig. 3). The radioactivity in the ATP pools differed slightly between pactamycin-treated and control embryos, apparently because DNA synthesis stops when protein synthesis is inhibited and this reduces the turnover of the ATP pool (Fig. 3, inset). The accumulation of radioactive RNA is not significantly different in normal and pactamycin-treated embryos; in both cases the RNA accumulates asymptotically to a steady state level in 5–6 h (ref. 2). The small difference between controls and treated embryos evident in Fig. 3 is the largest difference observed in any experiment. If synthesis is constant the decay curve is the inverse of the accumulation curve indicating that the decay of RNA must be essentially similar under both conditions. When the incorporation is initiated after a 4-h preincubation with pactamycin, the initial rate of accumulation of RNA is identical to controls, establishing that the instantaneous rate of RNA synthesis is not changed by pactamycin treatment. Therefore, the similarity in the accumulation

curves cannot be the result of pactamycin causing counterbalancing changes in the synthesis and decay of the RNA. About one-third of the total RNA accumulating in sea urchin embryos at this stage is hnRNA while the other two-thirds is mRNA. Since each of these two classes constitutes a significant portion of the total RNA, a change in synthesis or decay of either class would be readily apparent. It is clear that mRNA synthesis and decay remain normal when protein synthesis is inhibited by pactamycin.

Although the synthesis and decay kinetics of RNA did not change when initiation was inhibited by pactamycin, it was still possible that the transport of the newly synthesised mRNA to the cytoplasm was aberrant. In untreated embryos the percentage of radioactive RNA accumulating in the cytoplasm as mRNA increases asymptotically from a few per cent after a 15-min pulse to a steady state of 60% after several hours² (Fig. 4). Pactamycin had no effect on these kinetics of transport of newly synthesised mRNA to the cytoplasm (Fig. 4). As in normal embryos, the percentage of radioactive RNA in the

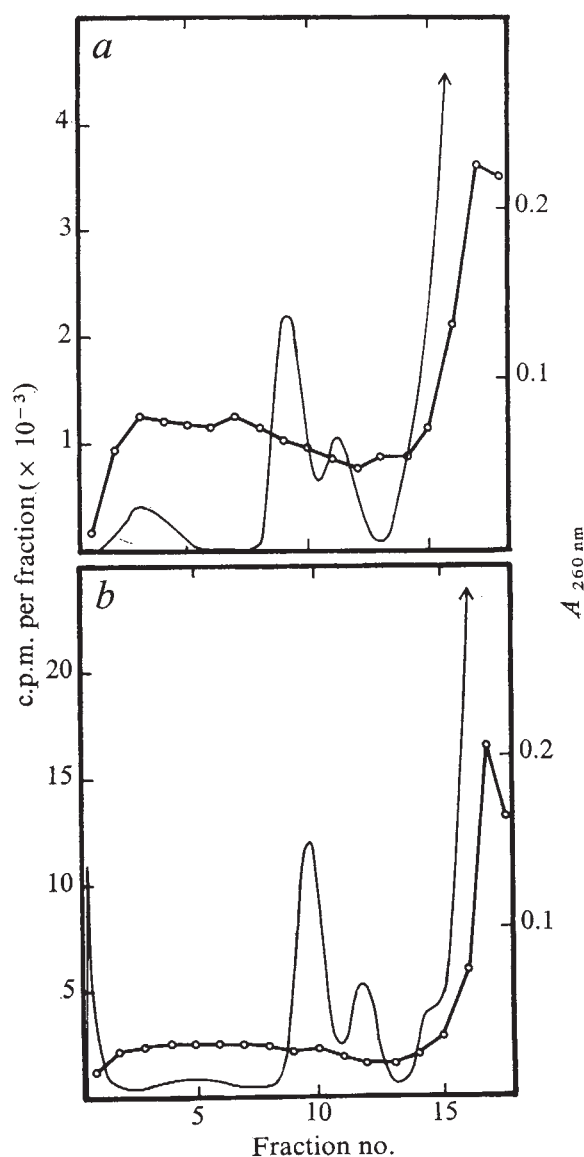


Fig. 2 Sucrose density gradients of the monosomes and ribosomal subunits. 5×10^4 Embryos were labelled and processed as described for Fig. 1. Cytoplasmic fractions were layered on sucrose density gradients and centrifuged for 15 h at 20,000 r.p.m. at 4°C, and fractions were processed as described. $4 \mu\text{g ml}^{-1}$ pactamycin and ^3H -adenosine were added concurrently for 30 min (a) and for 4 h (b). The solid line shows A_{260} .

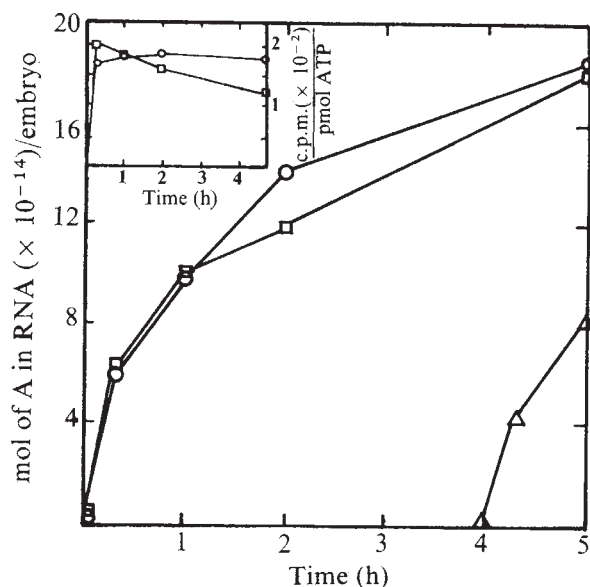


Fig. 3 Accumulation curves of radioactive RNA in the presence and absence of pactamycin. The methods for determining the accumulation curves for the synthesis of RNA have been described and justified previously². Briefly, embryos were labelled with ³H-adenosine, and a fraction was removed for RNA determination at each time point. Each fraction was homogenised in 0.5 N HClO₄ and the precipitate was collected by centrifugation and washed with 0.5 N HClO₄. The ATP was isolated from the acid extract of whole embryos, assayed by means of the luciferin-luciferase system, and counted for radioactivity. The acid-precipitable RNA was hydrolysed with NaOH and the alkaline-labile radioactivity in RNA and the alkaline-stable radioactivity in DNA were determined. The control sample (□) received no antibiotic; 4 μg ml⁻¹ pactamycin was added to one sample concurrently with the isotope (○) and to a second sample 4 h before addition of isotope (Δ). Inset: kinetics of incorporation of ³H-adenosine into ATP. The specific activity of the ATP pool was determined at each time point (see above). □, Control embryos; ○, pactamycin-treated embryos.

cytoplasm of pactamycin-treated embryos increased to about 60% in 4–5 h. This shows that mRNA is transported to the cytoplasm without associating with ribosomal subunits. Even though it accumulates there free of ribosomal particles and is not translated, it decays normally.

This normal synthesis, transport and decay of mRNA in the absence of protein synthesis indicates that translation is not

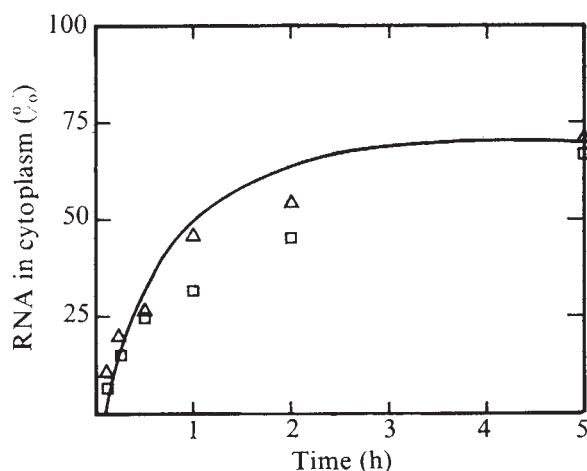


Fig. 4 Kinetics of transport of RNA to the cytoplasm. 10⁴ embryos were labelled and processed as described for Fig. 1 to yield nuclear and cytoplasmic fractions. Samples were hydrolysed in 0.3 N NaOH for 90 min, and the alkaline-labile radioactivity in RNA was determined. 4 μg ml⁻¹ pactamycin was added 2 h before label (Δ), or concurrently with label (□). The solid line shows the theoretical curve calculated from decay rates and steady-state levels of nuclear and cytoplasmic RNA².

involved in the mechanisms which control mRNA metabolism and that the proposed models that contain such mechanisms are not likely to be valid in sea urchin embryos. They also show that concomitant protein synthesis is not required for normal mRNA or hnRNA metabolism. If there are specific proteins normally associated with mRNA^{9,10}, these are either present in large excess, are recycled or have no role in regulating RNA metabolism.

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Binding of flexible ligands to macromolecules

MANY of the small molecules, such as enzyme substrates and inhibitors, hormones and neurotransmitters, the interactions of which with macromolecules are of fundamental importance in biology, may exist in solution in a number of conformations in equilibrium with one another. On the other hand, evidence from crystallographic studies suggests that when bound to a macromolecule these small flexible ligands have a single, well defined, conformation. The formation of such a ligand-macromolecule complex must therefore involve a process of conformational selection, which will influence the binding constant and the kinetics of complex formation. The apparent binding constant will simply be the product of the binding constant for the 'correct' conformation of the ligand and the mole fraction of this conformation in solution. The kinetic effects of conformational selection will depend on the mechanism of the binding process, and two contrasting models for this may be considered^{1,2}.

The first model is essentially an extension of Fischer's 'lock-and-key' model (Fig. 1a) in which only those ligand molecules which collide with the binding site in the correct conformation (and the correct orientation) are assumed to form a stable complex. In this single-step binding, all the favourable interactions between each 'segment' of the ligand and the corresponding 'subsites' on the macromolecules are formed simultaneously. The unfavourable losses of ligand entropy (translational, rotational and conformational) also occur in a single step. Thus the change in free energy on binding is given by

$$\Delta F = -RT \ln K = \sum_i \Delta H_i - T(\Delta S_{tr} + \Delta S_{rot} + \sum_i \Delta S_{conf,i})$$

(where ΔS_{tr} , ΔS_{rot} and ΔS_{conf} represent the changes in translational, rotational and conformational entropies of the ligand, ΔH_i represents the contribution to the enthalpic change from the binding of the i^{th} segment and the summation is over all 'segments' of the ligand). As for the kinetics of binding, in the absence of any orientational or conformational restrictions, we would expect the rate of binding to be controlled by diffusion. For the 'lock-and-key' model, however, we would expect the association rate to be less than this, as a given encounter will lead

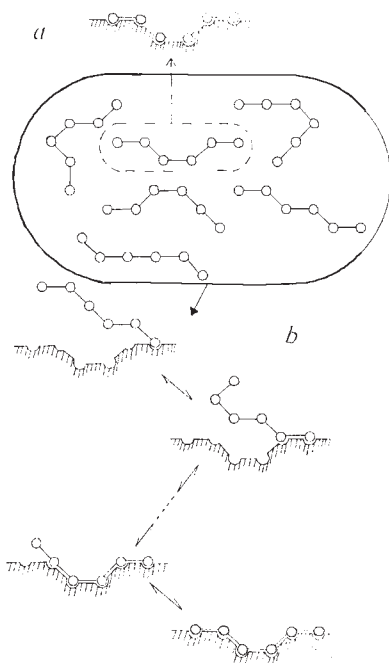


Fig. 1 Schematic representation of (a) 'lock-and-key' and (b) 'zipper' models for ligand binding discussed in the text. Only those ligand molecules which instantaneously have the correct conformation can bind as in (a), but essentially all ligand molecules can bind as in (b).

to stable binding only if the conformation of the ligand is suitable at that instant. In addition, this conformation must be presented in a suitable orientation, and this will further reduce the effective association rate constant. For a relatively large, flexible ligand, such as a peptide, this combination of conformational and orientational effects could easily lead to a reduction in the association rate constant by several orders of magnitude.

An alternative model is shown schematically in Fig. 1b. This model, which is similar to the 'zipper' model used in studies of double-helix formation in nucleic acids³⁻⁶, has been discussed previously⁷, and a detailed statistical-mechanical analysis of a distinct but related model has also been presented⁸. Here, we suppose that an initial, 'nucleation' complex can be formed by interaction of a single segment of the ligand with its subsite, and that this is followed by a series of conformational rearrangements of the partly bound ligand, leading to binding of the remaining segments to their appropriate subsites. The association rate constant for the formation of the initial complex will approach closer to the diffusion limit than that for the 'lock-and-key' model since the orientational and conformational requirements will be substantially less stringent. While the favourable enthalpy change on binding will be much less for this single-subsite interaction than for the binding of the whole ligand molecule, the loss of conformational entropy will also be considerably less (the changes in translational and rotational entropy being the same in both cases). Thus for the nucleation subsite

$$\Delta F_1 = -RT \ln K_1 = \Delta H_1 + T(\Delta S_{tr} - \Delta S_{rot} - \Delta S_{conf,1})$$

If this initial, single-subsite binding is to be followed by complete binding of the ligand to all the subsites, its lifetime must be long enough for the occurrence of the conformational rearrangements necessary for binding of an adjacent segment. Even if the lifetime of the complex is very short, say 10^{-7} s (corresponding to a binding constant $K \sim 10 \text{ M}^{-1}$ and an association rate constant $k_{on} \sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$), this is still long compared with the time required for rotation about single bonds, which may occur at rates greater than 10^{10} s^{-1} . For the subsequent propagation steps, again only a partial loss of conformational entropy is

involved and here there is no loss of translational or rotational entropy. For the binding of the n^{th} segment

$$\Delta F_n = -RT \ln K_n = \Delta H_n - T\Delta S_{conf,n}$$

The fact that a smaller entropy loss is involved in propagation than in nucleation leads to a substantial 'cooperativity' in the binding⁷. In other words, there is a strong probability that nucleation will be followed by propagation, leading to complete binding.

Attempts have been made to define the conformational distribution of small molecules such as acetylcholine in solution^{9,10}, on the grounds that this is an important parameter in considering their binding to a receptor. As a corollary of this argument, it has often been assumed that the conformation of the ligand in the complex corresponds to the predominant conformation of the ligand in solution, although there is no *a priori* reason why this should be so, and indeed there are a number of instances where it is not true¹¹⁻¹⁴. If the conformation adopted by the ligand in the complex is one which is poorly populated in free solution, the 'lock-and-key' model predicts that the association rate constant will be low. On the other hand, for the 'zipper' model, since the rate of binding is likely to be determined by the nucleation step, the forward rate will be less dependent on the nature of the bound conformation. The 'zipper' model thus provides a mechanism for the rapid binding of ligands, even when a conformation of low population is involved.

The kinetic difference between these two models for ligand binding can be simply represented in terms of energy barriers (Fig. 2). In the 'lock-and-key' model there is a single large energy barrier (free energy of activation) for binding, containing translational, orientational and conformational terms. In the

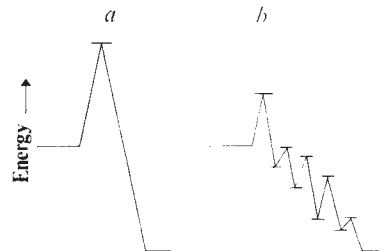


Fig. 2 Schematic representation of the 'reaction coordinate' for ligand binding by the 'lock-and-key' (a) and 'zipper' (b) models.

'zipper' model, this is replaced by a series of smaller energy barriers corresponding to the successive steps in binding, and since the overall rate is related to the height of the largest energy barrier in the reaction sequence, the association rate will be accelerated. As the two models only represent alternative pathways for going from the same initial to the same final state, the equilibrium constant must of course be the same for both models. Therefore, the faster association rate predicted by the 'zipper' model will be accompanied by a proportionately increased dissociation rate; again, only a series of relatively small energy barriers needs to be overcome.

The kinetic advantage of the 'zipper' model acquires additional interest when one considers the possibility of mutual conformational adjustment of both the ligand and macromolecule. In the 'zipper' model, this mutual adjustment can occur in a series of discrete, successive steps, between which the overall activation energy is partitioned. The overall process will thus, again, be significantly faster than in the 'lock-and-key' model. Since the activation energies for conformational changes in macromolecules may be considerable, a process which can lower these barriers may be expected to lead to a substantial acceleration of the overall rate of binding.

The two models presented here are obviously oversimplified and extreme cases. Behaviour intermediate between the 'lock-

and-key' and 'zipper' mechanisms is not only possible but likely. One might expect that molecules with limited degrees of conformational freedom would show behaviour closer to the 'lock-and-key' case, whereas very flexible ligands (such as linear oligopeptides) would bind in a 'zipper' fashion. There may also be cases where the energy barrier to a necessary conformational change is so high that the probability of its occurrence during the lifetime of the nucleation complex is very small¹⁵; the binding process in this case will tend to follow the 'lock-and-key' mechanism.

The development of the ideas presented here arose from our interest in the solution conformations of peptide hormones. These molecules seem to have a large number of conformations of similar energy, yet they are clearly capable of binding rapidly and specifically to a receptor. As pointed out by Eigen⁴, a mechanism for information transfer must satisfy the dual criteria of accuracy (specificity) and speed. High specificity is most rapidly achieved by having a relatively large recognition site (that is, a relatively large number of 'segments'). The 'zipper' mechanism affords a simple way to reconcile this requirement with that for rapid onset and offset of action.

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Cytoplasm-chromosome interactions in *Drosophila melanogaster*

INSTANCES of exceptionally high frequencies of mutation¹, male recombination², chromosomal aberration³ and sterility⁴ have been observed, often simultaneously⁵⁻⁸, in many natural populations of *Drosophila melanogaster* during the past thirty years. In some cases^{2,6,8}, these exceptional phenomena seem to be associated with chromosomes but not necessarily with specific

loci. Tests for an infectious agent have given negative results^{2,4}. Cytoplasmic factors, interacting with specific chromosomes, have been implicated in a few cases^{4,7} but in many others the experimental design did not provide for their detection. We report large differences in male recombination and F₁ sterility between reciprocal crosses of strains derived from temporally and geographically diverse natural populations and several commonly-used laboratory marker and balancer stocks. Cytoplasm-chromosome interactions are suggested.

The dictum that spontaneous crossing over does not occur in *D. melanogaster* males has been widely accepted since the early reports of Morgan⁹. Non-trivial frequencies are therefore indicative of unusual genetic events. We estimated the frequency of recombination in the third chromosome of F₁ males produced by mating males from each of twelve wild-type strains (Table 1) with females of the multiple-marked third chromosome stock *rucuca*¹⁰. The twelve strains are identified by the location and year of collection from the wild, if known. Since collection, the strains have been maintained by mass mating in half-pint milk bottles. Individual *rucuca* F₁ males were mated with five virgin *rucuca* females in 8-dm vials. Progeny emerging from the first 9 d of egg production were scored. Temperature was controlled at 25 ± 1°C and a standard maize-meal-molasses-agar medium, seeded with live yeast, was used.

F₁ male fertility was normal in all *rucuca* crosses. The mean minimum percentage recombination is a conservative estimate of the frequency of independent male recombination events in the total length of the chromosome because all clusters of multiple recombinants, within the same interval of individual males, are counted as single events. Non-trivial frequencies of male recombination were observed in chromosomes which had been derived from natural populations within the last decade but not in those from longer-established laboratory stocks, differences between individual strains of the two groups are as great as two orders of magnitude. Differences among strains within the high recombination group are generally smaller but significant ($P < 0.001$).

Five of the strains which were tested for male recombination in the third chromosome were also tested with a second chromosome marker stock, MMIIa. This stock is homozygous for the markers: aristaless, *al* (2-0.01); clot, *cl* (2-16.5); black, *b* (2-48.5); curved, *c* (2-75.5); speck-2, *sp*² (2-107.0). F₁ males for recombination tests were produced in two ways: in cross A, wild-type males were mated with MMIIa females and in cross B, MMIIa males were mated with wild-type females. Individual MMIIa F₁ males were, in each case, mated with five homozygous MMIIa virgin females and the frequencies of F₁ sterility and male recombination recorded (Table 2). In cross A all four strains which had exhibited male recombination in the third chromosome also did so in the second chromosome. Recombination in Oregon R-C was negligible in both chromosomes. In contrast, cross B male recombination was either absent or much reduced from the level observed in cross A. Moreover, F₁ fertility in both males and females was drastically reduced in cross A in the cases of Cranston, Harwich and Weymouth strains whereas, cross B fertility was normal in every

Table 1 Mean frequencies of sterility and third chromosome recombination in F₁ males from crosses between *rucuca* females and males from twelve wild-type strains

Strain	Male sterility %	Total progeny	Minimum male recombination %
Oregon R-C, 1925	3.3	4,372	0.02
Princeton, 1929	0	4,965	0.02
Ames I, 1938	0	2,522	0.04
Canton S	4.0	2,568	0.04
Nettlebed	3.3	3,295	0.03
Ottawa, Ontario, 1962	3.3	4,982	0
Cranston, Rhode Island, 1964	2.6	9,593	1.39
Amherst, Massachusetts, 1966	5.0	4,534	0.26
Harwich, Massachusetts, 1967	3.3	4,664	2.19
Chepachet, Rhode Island, 1974	0	2,400	0.70
Weymouth, Rhode Island, 1974	5.0	3,129	0.35
Winnepeaukee, New Hampshire 1974	3.2	2,157	0.93

Table 2 Mean frequencies of F_1 sterility and second chromosome male recombination from reciprocal crosses between the MMIIa marker stock and five wild-type strains

Strain	Cross A*				Cross B†			
	F_1 female sterility %	F_1 male sterility %	Total progeny	Minimum (male) recombination %	F_1 female sterility %	F_1 male sterility %	Total progeny	Minimum (male) recombination %
Oregon R-C, 1925	6.7	0	1,825	0	0	0	1,710	0
Cranston, Rhode Island, 1964	46.0	57.8	3,020	0.83	0	0	4,449	0.07
Amherst, Massachusetts, 1966	ND	6.8	3,504	0.46	0	0	3,221	0
Harwich, Massachusetts, 1967	68.3	41.7	3,731	1.13	4.2	5.0	1,802	0.11
Weymouth, Rhode Island, 1974	20.0	9.1	3,451	0.32	0	2.0	3,024	0.07

* MMIIa female $\times$ wild-type male.† Wild-type female $\times$ MMIIa male.

ND, not determined.

case. Sterility is measured as the percentage of F_1 individuals which produce no offspring when mated with three or more members of the opposite sex. This is a conservative estimate because many of the individuals which were classified as 'fertile' in cross A produced considerably fewer progeny than their counterparts in cross B.

Male recombination and sterility continued through at least two succeeding generations of suitable back-crossing, at levels undiminished from those in the F_1 . In each of three generations, males heterozygous for Cranston and MMIIa, were back-crossed with MMIIa virgin females and their progeny tested as previously described. The results (Table 3) suggest that male recombination and sterility arise from interactions between chromosomes of some strains and the cytoplasm of others, each combination possibly exhibiting its own unique characteristics.

Reciprocal differences in both male recombination and sterility are not restricted to crosses with the MMIIa stock. Strains which produced high male recombination in the third chromosome (Table 1) showed greatly reduced frequencies in reciprocal crosses with the *rucuca* marker stock: the numbers of independent male recombination events were 0 in 1,848 progeny with Amherst, 1 in 5,678 (0.2%) with Cranston and 9 in 3,623 (0.25%) with Harwich.

Although *rucuca* crosses gave progeny with normal fertility irrespective of the direction of the cross, matings of the Cranston strain with several other, widely-used, balancer and marker stocks have yielded large reciprocal differences in sterility: for example, with the multiple balancer stock H-41 (ref. 11), F_1 sterility was 35.8% in cross A and 0.8% in cross B; with the *Basc* marker stock, over 50% sterility was observed in $F_1 \times F_1$ matings. Picard *et al.*¹² observed seemingly similar interactions between strains, resulting in female sterility.

differences in the same direction.

These results may have importance, not only for the understanding of the aetiology of these exceptional phenomena but may also have implications for reproductive isolation between sympatric populations. Moreover, if cytoplasm-chromosome interactions between laboratory marker and balancer stocks and other strains prove to be common, greater care will be necessary in the design and interpretation of experiments using such stocks.

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Errata

In the article "Evidence for a Y chromosomal contribution to an aggressive phenotype in inbred mice" by M. K. Selmanoff, J. E. Jumonville, S. C. Maxson and B. E. Ginsburg (*Nature*, **253**, 529; 1975) the column headings in Table 1 should read DBA/1 Fathers and C57BL/10 Fathers respectively, in both studies.

In the article "Earthquake simulation by nuclear explosions" by O. C. Kolar and N. L. Pruvost (*Nature*, **253**, 242; 1975) the following corrections should be made. Under the heading 'Seismic monitoring' the first sentence of para. 3 should read, "P-wave trains from an earthquake are often extended in time whereas those from an explosion tend to be more compact." Under the heading 'Seismic evasion', para. 11, line 1 should read "... Fourier spectra of the composite of ...". Under the heading 'Practical considerations', para. 2, line 4 should read "operation; including the conceptual design of a ..." and para. 10, line 5 should read "... depth is in the range of 120W^{1/3} m (ref. 5)."

Table 3 Mean frequencies of sterility and male recombination in three generations of backcrossing Harwich 1967/MMIIa males to MMIIa females

Generation	Female sterility %	Male sterility %	Total progeny	Minimum male recombination %
1	68.3	41.7	3,731	1.13
2	57.1	51.2	1,870	1.07
3	47.5	49.2	3,270	1.01

In summary, we have observed non-trivial frequencies of male recombination in F_1 males of crosses between second and third chromosome marker stocks and wild-type strains which had recently been collected from natural populations. Longer-established laboratory stocks did not exhibit this phenomenon to any significant extent. In susceptible strains, male recombination seems to be dependent on the direction of the initial parental cross. In at least three crosses, male recombination and sterility were highly correlated, both showing large reciprocal

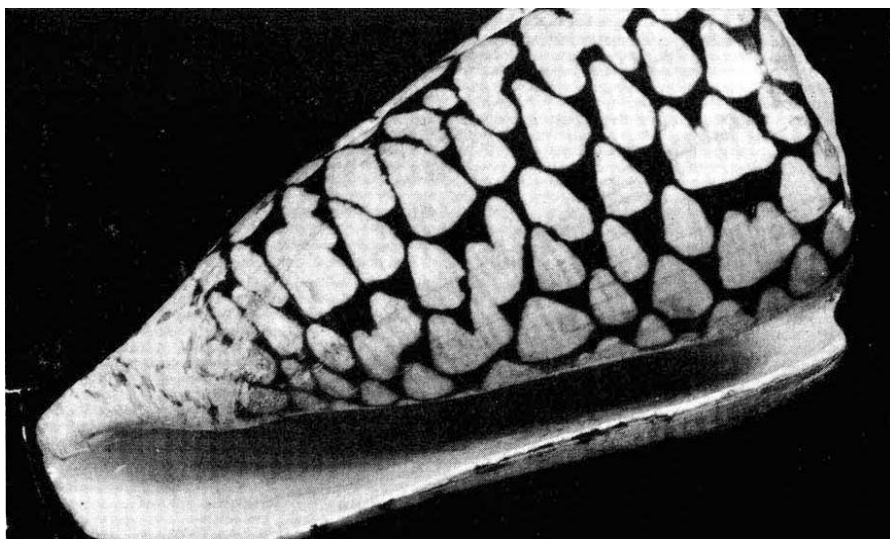
reviews

THE Mollusca are surely the most interesting of the invertebrate phyla. They are unique in producing a shell which may be considered independently of the animal which formed it, so that their study has been divided between conchologists and malacologists. True, the acumen of Aristotle enabled him to view the molluscs as a whole, apart from a not unnatural assignment of the cephalopods to a higher rung in his ladder of life, but this unity disappears among his successors.

Enlargement of the world at the renaissance led to the accumulation of shells from all oceans and it was these, beautiful in themselves, indestructible and eminently suitable for cabinet display, which occupied the attention of Martin Lister in the 17th century. Such names as he and others bestowed became incorporated into the binomial nomenclature of the *Systema Naturae*. But Linnaeus was content to group the animals into ten 'genera' and his followers, after describing the shell, merely referred "its animal" to one of these. All shelled species became included in the Testacea; only the shell-less slugs were classed as Mollusca which embraced a vast disorder of worms, zoophytes and echinoderms.

Malacology as such emerged from the comparative anatomical studies of Cuvier, which revealed the basic molluscan plan that is often ingeniously disguised because of the unique plasticity of the phylum. During the last century, as knowledge about the molluscan animal increased it was alongside a world wide search for shells described in numerous, often magnificently illustrated, shell iconographies.

This century has seen impressive progress in molluscan studies covering every aspect from population dynamics to the finest structural details revealed by the electron microscope. Of recent years there has been a revival of popular interest in shells, which has led to, and been further stimulated by, the publication of a series of impressive volumes, often with magnificent coloured plates, coming largely from the United States and Japan. Only rarely, however, as in Wilson and Gillett's *Australian Shells*, do animals themselves receive adequate attention. And



Suitable for cabinet display

it is with the laudable hope of remedying this lack of general knowledge about the living animal that Dr Alan Solem, one of the most rigorous contemporary malacologists whose activities are centred on, but far from confined to, the Field Museum of Natural History at Chicago, has written this appropriately named book*.

He should certainly succeed in his object. This is a very attractively produced and most excellently illustrated book which cannot but increase the knowledge and molluscan interests of readers ranging from the amateur conchologist to the professional zoologist. Naturally, there are inequalities in knowledge and presentation. The extraordinary plasticity of molluscs has been responsible for their unrivalled diversity of form and of habit. No one is likely to be equally conversant with limpets and squids and be as knowledgeable about the environment of abyssal seas as about that of deserts. Moreover, those who study marine molluscs seldom have time to concern themselves with terrestrial species, and the reverse is no less true.

Dr Solem is a member of the latter fraternity and in his chapters dealing with land molluscs, a preoccupying abundance of some 24,000 species, he writes with impressive authority. We encounter the fascinating devices for reproduction such as those in *Limax cinerioniger* which is five to eight inches long but in which the genitalia, protruded by the intertwining pair, reach

lengths of up to 32.5 inches; he also covers adaptations for survival in desert climates or over long dry periods, and the course of evolution, as usual in molluscs from many converging origins, of the land slugs which have largely ceased to be 'shell makers'. There is an altogether admirable chapter dealing with how "To scrape a Living", concerned with the radula and illustrated with 21 plates including scanning electron microscope photographs that reveal the structural diversity of this wonderfully adaptable tool which has been so very significant in the success of the phylum.

Dr Solem has been too deeply preoccupied with land mollusca and snails to have the same commanding knowledge of marine species. He does not attempt to describe the ctenidial gills, organs as supremely successful as the radulae which, indeed, they supercede as organs of feeding throughout the Bivalvia in which group, it must be noted, the palps do not (as is claimed in the book) push food into the mouth. Nor can I follow Dr Solem in his treatment of the vexed problem of torsion.

But this is a revealing book for all readers, enriched by appendices covering classification, references for further reading, a glossary, and some excellent advice about the care and feeding of both marine and freshwater molluscs, and of land snails and slugs. In all, a most welcome addition to molluscan literature.

C. M. Yonge

**The Shell Makers*. By G. A. Solem. Pp. 280. (Wiley: London and New York, June 1974.) £5.05.

Instant zoogeography

Aspects of Zoogeography. By Paul Müller. Pp. viii+208. (Junk: The Hague, 1974.) Dfl. 35.

PAUL MÜLLER is known for some interesting work on Brazilian reptiles and the zoogeography of South America. His latest work, entitled *Aspects of Zoogeography*, runs briefly through definitions of zoogeography and the biosphere. It summarises the characteristics of zoological realms, there are brief sections on each of nine terrestrial and two freshwater biomes. It contains a trendy little chapter on urban ecosystems. And the book ends briefly with historical aspects of zoogeography. All this in 170 pages, half of them occupied by illustrations.

It is difficult to see how some of the diagrams are related to the text and many of the diagrams are difficult to understand because they are too complex or the captions fail to describe them.

In the brief text, there is little discussion. For example, continental drift is mentioned but its relevance to animal distribution is not considered. The desert lizard *Uromastix* is listed as having salt glands but the significance of salt excretion for desert lizards is not discussed.

The book could be useful to a student

who wants a synopsis of zoogeography. The references are there and the bibliography lists German works in addition to more familiar English and American works.

But the book is unsatisfactory because there is no assessment of the facts; and no personal opinion emerges, except for the assertion that the dispersal centre concept of Reinig and de Lattin should be given more attention. But it would be necessary to turn to de Lattin's work to discover the implications of that.

The book has been incompetently edited. There is no excuse for so many misprints.

Wilma George

Colour and function of pigments

The Significance of Zoochromes. By A. E. Needham. Pp. xx+429. (Springer: Berlin and New York, 1974.) \$26.50.

THE pioneer investigations of natural pigments by C. A. MacMunn at the end of the last century did not stimulate much interest among his contemporaries, and it is only within the last 25 years or so that his work has received the attention it deserves. Even now, however, researchers who have their main interest in pigments in their own right form a small and exclusive band.

Dr Needham's book is doubly welcome, for it not only considers animal pigments from the points of view of structure and function, but it also presents information and concepts which have never been assembled in one volume before. A discussion of the molecular basis of colour and the chemistry of the natural pigments is followed by the main part of the book which deals with the functions of these pigments. Biochemical and physiological rôles are considered, leading to an assessment—supported by evidence from metabolic pathways, from evolution and from the genetic basis of pigment formation—of the significance of the pigments in animals.

The format of the book is very pleasing, with particularly high quality typeface and paper. It was a good idea to preface each chapter with a brief synopsis of its subject matter and to include a conclusion section at the end. There are many tables which collect data not easily found elsewhere: the discussion of the taxonomic distribution of pigments with its accompanying table is a good example. The chapters dealing with integumental pigments and camouflage and the biogenetic evidence for their significance are of great interest, and the final chapter, a general assessment, contains

much that is controversial. The list of references is comprehensive and most useful.

Unfortunately, the book contains many errors and misguided attempts to change well-tried and familiar nomenclature, which will make it irritating to those who have spent many years in this field. The title itself is provoking—why not 'Animal Pigments'?—'pigment' is a much better word than the rather pompous 'chrome', which, in any case, suggests the metal. To use the word 'porphyran' for 'metalloporphyrin' is misleading (*malgré* Fearon), and suggests a false relationship with porphyrin. The words 'glycose' and 'alkanol' are based on false logic. Use of the word 'chromosome' to describe a pigment granule leads to a hopeless confusion, both written and spoken. There is a daunting list of quite superfluous abbreviations at the beginning of the book.

Straightforward errors are more serious, and space allows mention only of the very worst. The use of the word 'chloroporphyrin' when 'chlorocruoroporphyrin' is meant is an illustration of the way in which obstinate pedantry reaps its own reward. Svedberg divided invertebrate haemoglobins into erythrocruorins and chlorocruorins for excellent and sound reasons, and it is chlorocruorin which gives rise to chlorocruorohaem and chlorocruoroporphyrin. Chloroporphyrin is a tricarboxylic derivative of chlorophyll. Phthalocyanins do not contain indole nuclei but are tetrazaporphyrins in which the methene bridges are replaced by tertiary N and the four pyrrol rings are condensed with benzene rings in their β -positions. Fossil porphyrins do not always lose their COOH groups. Solubility data, fluorescence, and the formulae for many porphyrins are often wrong. The important feature of the Soret band in the porphyrin absorption spectrum is that it is dependent upon the integrity of the macrocyclic ring (not "super-ring").

There are many omissions of important material which one would expect to find in a book of this kind. They include Warburg's ideas about primitiveness and the formyl group; mention of the pigments of birds' eggshells; the photodynamic work of Meyer-Betz, Boyd and Gaffron with porphyrins and proteins; and discussion of the effects of substituent groups and metals on the porphyrin absorption spectrum.

The author has striven too hard to achieve absolute exactness, and this has led him into pedantry and often downright error. Even as it stands, however, the book is a valuable and unique contribution to the literature on pigments.

G. Y. Kennedy

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Sweet review

Sugars in Nutrition. (The Nutrition Foundation: A Monograph Series.) Edited by H. L. Sipple and Kristen W. McNutt. Pp. xx+768. (Academic: New York and London, November 1974.) \$45.00; £21.60.

THIS powerful and informative book is based on a number of papers presented at the International Conference on sugars held at the Vanderbilt University School of Medicine in 1972, at which world authorities within the field were very well represented.

The book commences with an historical account and general synopsis of sugars and nutrition, along with psychological and biochemical descriptions of their sweetness. A useful account of their occurrence and incorporation in foods is followed by some statistics of their use and recent technological advances. An entire section on the metabolism of sugars includes also some effects attributable to polyols, especially sorbitol and xylitol which are now so important as food additives and which are still, of course, items of modern therapy. This section includes a valuable paper from Macdonald on the metabolic effects of maltose and higher oligosaccharides, which are now being increasingly used by food manufacturers as a substitute for sucrose. A large section on disorders of sugar metabolism includes papers on inborn errors, sugar cataract, intolerance, hypertriglyceridaemia, cardiovascular disease and diabetes, and a section on therapy naturally includes some special emphasis on the uses and dangers of xylitol and mannitol in different forms of treatment. Finally, a section on the oral cavity deals with sugars, dental plaque and oral streptococci.

It is inevitable that a book of this magnitude should possess one or two puzzling features. Some contributors, for example, could have been expected to cover areas which have in fact been covered by different authors. Shallenberger, who has made one of the most radical contributions to the theory of sweetness in the last decade does not write either of the two papers on that subject. Nor is the major part of his work alluded to by the other two authors. It is, however, evident that some speakers at this symposium presented more than one work and that not all of these were selected by the editors.

Some of the newest and less well known research into sugars and nutrition does not seem to have been included in the book. Examples include the research by the National Aeronautics and Space Administration into the conversion of human waste products to formose sugars and their metabolic fate after re-feeding, and the

work at MIT on carbohydrates and brain metabolism. The effects of sugars on human work performance and fuel reserves seem also to have been excluded from discussion.

Any multidisciplinary scientific volume is liable to have its share of editorial mistakes and omissions, but of particular gravity are those on pages 153 and 155; the pyranose sugar structures depicted there are not the sugars they are claimed to be. Furthermore, the diagram of transport across a biological membrane gives the completely erroneous impression that D-glucose enantiomerises in the process. In spite of these faults, however, the book remains the best work to date for co-ordination and summary of the status of sugars in modern nutrition and physiology.

G. G. Birch

Sizing it up

Particle Size Analysis. (Series in Analytical Chemistry) By Z. K. Jelinek. Pp. 178. (Ellis Horwood: Chichester, 1974.) £5.00.

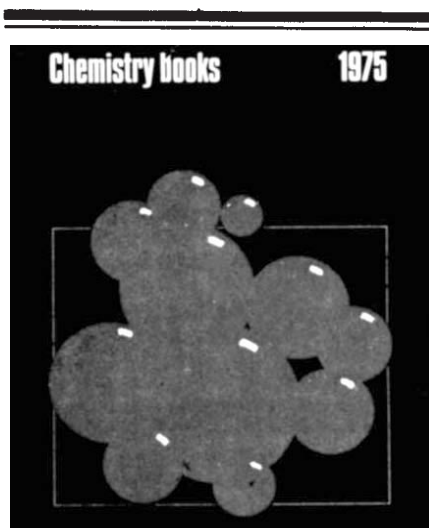
THE quantitative determination of the size, shape and distribution of particles in disperse systems is of considerable importance to the study of fogs and smokes, foams and emulsions, suspensions, porous solids and catalysts, and in general to systems consisting of a disperse and a continuous phase.

Particle sizes may vary from those found in polymer and colloidal solutions to those found in coarse dispersions, and as a consequence, a range of methods is necessary for the analysis of the different systems. This book gives a comprehensive account of the practical aspects of size analysis in these systems, and describes in detail the methods of measurement of size, shapes and pores in disperse systems, the measurement of surface areas by adsorption methods, and molecular weight measurements in polymer and colloidal solutions.

The coverage of the various methods is thorough, and the volume includes separate chapters on optical procedures (electron microscopy and ultramicroscopy, light scattering and X-ray diffraction), mechanical methods, gravitational and osmotic methods, viscosity and permeation, pore size analysis, and adsorption and conductometric measurements. The apparatus, technique and procedure is given in detail for each method, followed by a valuable selection of worked out examples. The references cover literature up to 1972.

This is a valuable reference work for chemists who desire a survey of the practical laboratory method of particle size analysis of dispersions.

C. E. H. Bawn



Molecular Behaviour and the Development of Polymeric Materials

Edited by A. LEDWITH and A. M. NORTH
January 1975: 562 pages: illustrated: 412 12400 9:
hardback: £12.00

This book opens with articles on the nature of ionic polymerizations and on the recently characterized role of intermolecular complexes in determining the course of polymerization reactions. Succeeding chapters consider topical aspects of general and specific applications of the main classes of polymers, and the book concludes with five reviews which discuss macroscopic physical and mechanical properties in the light of recent studies in molecular behaviour.

Growth of Crystals from the Vapour

M. M. FAKTOR and I. GARRETT
December 1974: 310 pages: illustrated: 412 11320 1:
hardback: £7.00

The text of this book starts with an introduction to crystal growth, followed by preparatory chapters on the thermodynamic basis of chemical vapour transport, and a discussion of crystallization and nucleation. Transport in the gas phase is then discussed from a simple theoretical standpoint. Interplay of transport and surface kinetic limitations and their influence on interfacial stability and electronic properties are explored next. The final chapters include a review of recent experimental exploration of some interesting concepts, and suggestions of where further effort could be applied.

Chromatographic Methods

Third edition
R. STOCK and C. B. F. RICE
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This new edition has been thoroughly updated incorporating the many new advances in the subject which have been developed since 1967. An attempt has been made to give some guidance as to the choice of method for a particular purpose and to assess the relative values of the different procedures.

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Multiplication from a statistical viewpoint

Mathematical Models of Conception and Birth. By Mindel C. Sheps and Jane A. Menken. Pp. xxiii+428. (University of Chicago: Chicago and London, 1973.) £9.25.

In the last two decades a new approach has thrown light on the biological factors underlying the statistics of human reproduction. It is an approach which involves considering a sequence of stages in the reproductive process (such as a period of susceptibility to conception, pregnancy, or post-partum sterility).

Mathematical models play an important role in these studies mainly because such models offer a way of dealing with quantities which are not directly measurable; for instance, the probability of conception in one menstrual cycle, or, to take an even more intractable example, the distribution of such probabilities over populations of women. By using mathematical models and computer simulation, it is possible to relate assumptions about quantities not accessible to direct measurement to observable quantities

such as birth rates, intervals between births and so on. The study of such models produces a number of surprising results. For example, contraception which reduces the probability of conception among women at risk by $x\%$, will not reduce the number of births by $x\%$, but by much less. Thus, an average contraceptive effectiveness of well over 60% will probably be required to reduce births by 40%.

The work under review is a careful, detailed, clear and authoritative survey of this fast expanding subject. It is fully up to the high standards set over three decades by the publications of the Princeton Office of Population Research. The work is published as a posthumous memorial to the senior author, Mindel Sheps, who made fundamental contributions in this field of research. She died just before publication of the book.

The book offers more than the title promises. The mathematical analyses of a great variety of models are discussed. Extensive tables are given, showing the results of varying the parameters which enter into the models. This kind of material was to be expected. In addition, however, the book contains expositions of the parts of probability theory on which the models are based. The reader unacquainted with renewal theory, Markov chains or Markov renewal processes will find an introduction here. There is also coverage of basic distribution theory and various mathematical techniques so that in principle a reader equipped only with calculus and a basic course in mathematical statistics could use this book. In effect, it may be viewed as a text of much of applied probability theory (and some statistical theory) with detailed applications in one special area. As befits a mathematical textbook, there are worked examples and exercises for the reader at the ends of chapters, with answers at the back of the book.

What the reader will not find (with occasional exceptions) are examples of real data to the analysis of which these models can be applied. For example, a long chapter is devoted to models of the intervals which elapse between marriage (or some other suitable initial point of observation) and the first conception or first birth. The authors justly remark, at the beginning of this chapter, that "data on conception times and related phenomena have been used a great deal both in investigations of the biological basis of reproduction and in efforts to evaluate the effectiveness of contraception". Yet not a single recorded distribution of intervals to the first conception or birth is actually shown, nor are references given to publications where such data may be found.

Paradoxically, this lack of contact with reality may increase the sale of the book. For it can probably be used in courses on applied probability theory and stochastic processes given by lecturers with no experience in analysing demographic data. Common interest in, and common-sense knowledge of, sex and reproduction are all that is required.

The book may strengthen the suspicion (which is unjustified in this case) that probabilistic models are the toys of mathematicians and computer specialists not seriously concerned with the phenomena on which the models are expected to throw light. **J. Hajnal**

Molecular collisions

Molecular Collision Theory. (Theoretical Chemistry: A Series of Monographs.) By M. S. Child. Pp. 300. (Academic: London and New York, September 1974.) £8.50; \$22.00.

In recent years rapid progress has been made in the experimental investigation of molecular scattering. These experiments are a very important source of information about the potential surfaces on which it is supposed that nuclei move. Molecular collisions may conveniently be classified into three broad classes—elastic, inelastic and reactive. Each class is sensitive to a particular aspect of the potential surface. This book is intended as an introduction to the theory required for the interpretation of elastic, inelastic and reactive scattering experiments in chemistry. Emphasis is placed on the analytical treatment of quantum mechanical and semiclassical aspects of molecular scattering.

Chapters 2–5 present the quantum-mechanical and semiclassical theories of elastic scattering. They include such important topics as the Born approximation, the semiclassical phase shift, rainbow and glory scattering and orbiting. The theory of inelastic scattering is expounded with the aid of the scattering matrices in the next four chapters. The final chapter is devoted to reactive scattering. There are five appendices on relevant mathematical topics, including continuum wavefunctions and classical mechanics.

In the selection of material emphasis has been placed on obtaining analytical expressions of practical value to those involved in interpreting chemical experiments. The book is very successful in this respect and is likely to be popular with research students and others. It is authoritative, penetrating and compact. Although there are a number of minor errors I strongly recommend the book. **A. D. Buckingham**

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obituary

Robert Robinson was born in 1886 at Chesterfield in Derbyshire and went to the University of Manchester to study chemistry, where he came under the influence of W. H. Perkin, Jr, a brilliant organic chemist then at the height of his powers and in charge of a famous research school; his Manchester contemporaries included W. N. Haworth, J. F. Thorpe, J. L. Simonsen and Chaim Weizmann who became his lifelong friends. Robinson became a colleague and close friend of Perkin and they collaborated in a series of beautiful researches on the dyewood colouring matters, brazilin and haematoxylin, and on alkaloids. When only twenty-six years old he was appointed to the chair of organic chemistry in the University of Sydney, a position he held for three years until appointed professor at Liverpool in 1915. During his short stay in Sydney, and despite the upheaval of moving across the globe and the complication of a disastrous laboratory fire which destroyed many of his records, Robinson pushed ahead with his work on alkaloids and especially with his ideas on biogenesis based on structural relationships in the alkaloid group. His theories were given a tremendous boost by his synthesis of the alkaloid tropinone by the interaction, in dilute aqueous solution at room temperature, of three simple molecules all of which could well be plant metabolites. His paper on tropinone published in 1917 not only led to widespread adoption of his biogenetic theories as a tool in natural product studies, but provided the primary stimulus to all the modern developments in the elucidation of biosynthetic mechanisms.

Colour in nature had fascinated him from the time of his early work on the dyewoods, and, perhaps spurred on by the fact that he was a keen gardener, he turned his attention to the anthocyanin pigments of flowers. Already a master of the classical degradation methods of the organic chemist, he introduced a new approach in his anthocyanin studies—the use of analogue synthesis as a powerful tool in structure determination—which influenced the pattern of much future work in the natural product field. The total synthesis of all the major anthocyanins allowed him to initiate work on the genetics of flower colour variation; it also provided chemists with some new synthetic methods which, together with many others he devised and exploited during



Sir Robert Robinson

An appraisal by Lord Todd

his career, have greatly enriched the synthetic chemist's armoury of weapons.

His natural product work and especially his biogenetic theory have had a great and lasting impact. Equally vital to the development of organic chemistry, is his work on the electrochemical theory of organic reactions. Robinson's ideas on the nature of reactivity in organic compounds were stimulated and reinforced by his association with Lapworth in Manchester during the 1920s, and it would be fair to say that what is often called the Lapworth–Robinson theory of organic reactions expressed by them in terms of the electronic theory of valence, and powerfully developed by Robinson, provided an essential stimulus to all the great advances made since that time in our understanding of reaction mechanisms in organic chemistry. The electronic theory developed, however, when ideas of atomic structure were still rather primitive, but Robinson's views, re-interpreted in terms of modern molecular orbital theory remain valid to this day.

All these contributions were made during a career in which he occupied in quick succession five chairs of organic chemistry (Sydney, Liverpool, St Andrews, Manchester and University College, London) and one research directorship in industry (British Dyestuffs Corporation 1920–21) before he succeeded his old teacher Perkin as Waynflete Professor at Oxford in 1930,

a position he occupied until his retirement in 1957. Although his stay in industry was short, he made a profound impact not just on the British Dyestuffs Corporation but throughout his career, as consultant and adviser, on the chemical and pharmaceutical industries; he joined the Shell Chemical Company after his retirement from Oxford and continued to be associated until his death. Even though failing physically, he maintained the amazing clarity of mind for which he was famous.

As would be expected, honours were heaped on him by universities and learned academies all over the world. He served as President of the Chemical Society (1939–41), of the British Association for the Advancement of Science (1955) and of the Royal Society (1945–50). He was awarded the Nobel Prize for Chemistry in 1947 and for his services to science in Britain he was knighted in 1939, receiving the Order of Merit in 1949. He was twice married—his son and daughter, and his second wife, survive him.

His career would seem to leave little time for other activities. But Robert Robinson was no ordinary man. Although deflected from it at university he maintained a lifelong interest in mathematics. Perhaps related to this was his passion for chess of which he was a first-class exponent, having the entrée to the major chess-clubs in any city which he visited both here and abroad. He was also fond of music and was a much better pianist than he would admit. Out of doors he found recreation in mountain climbing and walking and spent his summer vacations traversing the Alps of which he had an encyclopaedic knowledge. A man of great physical and mental toughness he was nevertheless a shy man whose manner with strangers could sometimes be for this reason brusque or withdrawn. To those who knew him he revealed himself as he really was—kindly, generous and loyal. Humble in the face of nature, he loved children and perhaps for that reason was loved by them. He enjoyed the cut and thrust of scientific argument but he could on occasion be a little brusque with, or—which was more devastating—simply ignore those who were, in his view, being obtuse. Few men have inspired greater respect and real affection among those who were privileged to work under his guidance and to gain his friendship. With his passing Britain and indeed the world has lost one of the greatest men of science of this century.

announcements

Awards

Hollis D. Hedberg has been awarded the **Wollaston Medal** by the Geological Society for contributions to micro-palaeontology, stratigraphy and petroleum geology.

John Sutton has been awarded the **Murchison Medal** by the Geological Society for contributions to pre-cambrian geology, particularly in the Scottish Highlands and Islands.

Dorothy Raynor has been awarded the **Lyell Medal** by the Geological Society for contributions to vertebrate palaeontology and carboniferous geology.

Drummond H. Matthews has been awarded the **Bigsby Medal** by the Geological Society for his part in the Vine-Matthews hypothesis.

For his contribution to elementary particle physics, **Nicholas Kemmer** has been awarded the **J. Robert Oppenheimer Memorial Prize** by the Center for Theoretical Studies, Miami, Florida.

Appointments

Clifford Butler has been appointed Vice-Chancellor of Loughborough University of Technology.

Norman Blackburn has been appointed to the chair of pure mathematics in the University of Manchester.

Miscellaneous

Roussel Prize. Applications are invited for the Roussel Prize, awarded every two years to stimulate pharmaceutical research. The next prize (\$10,000) is scheduled for June, 1976 and will be concerned with work published before December, 1975 on steroids and related compounds. Nominations with the names of two referees, must be submitted before March 1, 1976. Further information from: The Secretary, Centre de Recherches, Roussel Uclaf, 93230 Romainville, France.

Theoretical Chemistry. The Charles Coulson Summer School in Theoretical Chemistry will be held at Oxford University from September 7-20, 1975. For further details, contact: Dr M. S. Child, Theoretical Chemistry Department, 1 South Parks Road, Oxford OX1 3TG, UK. Closing date: May 15, 1975.

Person to Person

Philosophy of Medicine. Contact wanted with people working in philosophy of medical science, especially the concept of disease, cause and the relation between the foundations of medical science and the health system. We are teaching a course 'The Theory and Philosophy of Medicine' at the University of Copenhagen. For information contact: Anders Ottar Jensen, Department of Biophysics, Juliane Maries Vej 28, DK 2100 Ø, or Hans Siggaard Jensen, Department of Philosophy, Købmagergade 50, DK 1150 K, Denmark.

Reports and publications

Great Britain

Energy Conservation in the United Kingdom: Achievements, Aims and Options. (A report by the National Economic Development Office.) Pp. viii + 106. (London: HMSO, 1974.) £3.40. [21]
 Bulletin of the British Museum (Natural History). Zoology, Vol. 27, No. 7: A New Family, Genus and Species of Bat (Mammalia: Chiroptera) from Thailand. By J. E. Hill. Pp. 301-336. (London: British Museum (Natural History), 1974.) £2.10. [31]
 Centre for Overseas Pest Research, Report, January-December, 1973. Pp. vi + 141. (London: Centre for Overseas Pest Research, College House, Wrights Lane, W8, 1974.) £1.30. [61]
 Science Research Council. Telecommunications. Pp. 24. (London: Systems Engineering Committee Secretariat, Science Research Council, State House, High Holborn, WC1, 1974.) gratis [81]
 A Park System for Scotland. Pp. 36. (Battley, Redgorton, Perth: Countryside Commission for Scotland, 1974.) [91]
 Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 269, No. 900: A New Genus and Species of Anthracoxaur Amphibian from the Lower Carboniferous of Scotland and the Status of *Pholidogaster pisciformis* Huxley. By A. L. Panchen. Pp. 581-640. (London: The Royal Society, 1975.) UK £2.40; Overseas £2.60. [91]
 Broadcasting: The Developing Technology. By James Redmond. (BBC Lunch-Time Lectures, Ninth Series, 2.) Pp. 20. (London: BBC, 1974.) [141]
 New Science in the Solar System. (A New Scientist Special Review. (Pp. 64 (55 plates). (London: New Science Publications, 1975.) £1. [141]
 National Radiological Protection Board. NRPB-R27: Factors for Deriving Absorbed Dose rates in Air due to Beta Particles from Measurements of Absorbed Dose-rates in Tissue-equivalent Material. By T. M. Francis and E. A. Pook. Pp. 12. NRPB-R28: The Use of Plutonium-239 Sources in Schools and Other Educational Establishments. By T. G. Williams. Pp. 18. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1974.) [151]
 Cancer Biochemistry Biophysics, Vol. 1, No. 1, 1974. Pp. 1-56. Edited by Harry Darrow Brown, in association with Robert C. Hahn. Subscription Rates (per volume, post paid). Six issues per volume, published bi-monthly. Great Britain: Personal subscribers who warrant that the journal is for their own use, and order direct from the publisher £6. Librarian, Institutions and other subscribers £22.75. (London and New York: Gordon and Breach Science Publishers, Ltd., 1974.) [161]
 Whale Campaign Manual 2. Pp. 132. (London: Friends of the Earth, 9 Poland Street, W1, 1975.) 80p plus 20p postage. [161]
 The Commonwealth Forestry Institute, University of Oxford. Fiftieth Annual Report, 1973/1974. Pp. 40. (Oxford: The Commonwealth Forestry Institute, The University, 1974.) [201]
 Freshwater Biological Association. Scientific Publication No. 29: Turbulence in Lakes and Rivers. By I. R. Smith. Pp. 79. (Ambleside, Westmorland: Freshwater Biological Association, 1975.) £1. [201]

Other countries

World Health Organization. Manual on Radiation Protection in Hospitals and General Practice. Vol. 1: Basic Protection Requirements. By C. B. Braestrup and K. J. Vikterlof. Pp. 81. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 12. [1812]
 Societ Biomedical Institutions: a Directory. (A Publication of the Geographic Health Studies Program of the John E. Fogarty International Centre for Advanced Study in the Health Science.) DHEW Publication No. (NIH) 74-698. (Washington, DC: US Government Printing Office, 1974.) \$5.95. [1812]
 Annals of the South African Museum. Vol. 66, Part 4: Type Specimens of Decapoda (Crustacea) in the Collection of the South African Museum. By Brian Kensley. Pp. 55-80. R.4. Vol. 66, Part 5: The Cretaceous Stratigraphy of South-Central Africa. By Michael R. Cooper. Pp. 81-107. R.4.30. Vol. 66, Part 6: The Zoogeography of the South African Avifauna. By J. M. Winterbottom. Pp. 109-149. R.7.50. Vol. 66, Part 7: Pedi Skin Dressing Technique. By E. M. Shaw and H. E. Bohme. Pp. 151-167. R.4.40. Vol. 66, Part 8: A New Species of Mystacocarida (Crustacea) from Alaga Bay, South Africa. By A. McLachlan and J. R. Grindley. Pp. 169-175. R.2.10. (Cape Town: South African Museum, 1974.) [1912]
 Smithsonian Contributions to Zoology. No. 181: The Costate Species of *Colaspis* in the United States (Coleoptera: Chrysomelidae). By Doris H. Blake. Pp. iii + 24. 65 cents. No. 182: Spider Crabs (Crustacea: Brachyura: Majidae) from the International Indian Ocean Expedition, 1963-1964. By D. J. Griffin. Pp. iv + 35. 85 cents. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [2312]
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 Centre National pour l'Exploitation des Océans (CNEOX). Publications, Serie: Actes de Colloques. Colloque sur l'Aquaculture, 22-24 Octobre, 1973, Brest. Pp. 472. Serie: Rapports Scientifiques et Techniques. No. 18: Cartographie Mensuelle des Données sur l'Effort et les Prises de la Pêcherie Palangrière Thonière Japonaise de l'Océan Atlantique, 1956-1971. Par J. Yves Le Gall. (Brest: CNEOX, 1974.) [2312]
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 The Art of Managing the Environment. (A Ford Foundation Report.) Pp. 42. (New York: Ford Foundation, 320 East 43 Street, 1974.) [3012]
 United States Department of the Interior: Geological Survey. Professional Paper 845: Ammonites from the Navesink Formation at Atlantic Highlands, New Jersey. By William A. Cobban. Pp. iii + 21 + 11 plates. (Washington, DC: Government Printing Office, 1974.) \$1.35. [3012]
 National Research Council Canada. Annual Report on Scholarships and Grants in Aid of Research, 1973/74. Pp. xi + 560. (Ottawa: Research Council Canada, 1974.) \$2.50. [3012]
 The American Journal of Drug and Alcohol Abuse, Vol. 1, No. 1, 1974. Pp. 1-140. Subscription rate for Vol. 1 (3 issues). \$25 + \$2.55 postage outside USA. (New York: Marcel Dekker, Inc., 1974.) [3012]
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